

A5366

**Selective Estrogen Receptor Modulators to Enhance the Efficacy of Viral
Reactivation with Histone Deacetylase Inhibitors**

A Multicenter Trial of the AIDS Clinical Trials Group (ACTG)

**Sponsored by:
National Institute of Allergy
and Infectious Diseases**

**Industry Support Provided by:
Merck Research Labs**

IND #

**The ACTG CURE Transformative
Science Group:**

Rajesh Gandhi, MD, Chair

Protocol Co-Chairs:

**Rajesh Gandhi, MD
Eileen Scully, MD, PhD**

Protocol Vice-Chair:

Daniel R. Kuritzkes, MD

DAIDS Clinical Representative:

Catherine Godfrey, MD

Clinical Trials Specialist:

Lara Hosey, MA

**FINAL Version 1.0
December 8, 2017**



A5366

Selective Estrogen Receptor Modulators to Enhance the Efficacy of Viral Reactivation with
Histone Deacetylase Inhibitors

SIGNATURE PAGE

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable US Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

Principal Investigator: _____
Print/Type

Signed: _____ Date: _____
Name/Title

TABLE OF CONTENTS

	Page
SIGNATURE PAGE	2
SITES PARTICIPATING IN THE STUDY	5
PROTOCOL TEAM ROSTER	6
STUDY MANAGEMENT	9
GLOSSARY OF PROTOCOL-SPECIFIC TERMS	12
SCHEMA	13
1.0 HYPOTHESIS AND STUDY OBJECTIVES	15
1.1 Hypothesis	15
1.2 Primary Objectives	15
1.3. Secondary Objectives	15
1.4. Exploratory Objectives	15
2.0 INTRODUCTION	16
2.1 Background	16
2.2 Rationale	17
3.0 STUDY DESIGN	23
4.0 SELECTION AND ENROLLMENT OF PARTICIPANTS	24
4.1 Inclusion Criteria	24
4.2 Exclusion Criteria	26
4.3 Study Enrollment Procedures	27
4.4 Co-enrollment Guidelines	29
5.0 STUDY TREATMENT	29
5.1 Regimens	29
5.2 Administration	29
5.3 Study Product Formulation	30
5.4 Pharmacy: Product Supply, Distribution, and Accountability	30
5.5 Concomitant Medications	31
6.0 CLINICAL AND LABORATORY EVALUATIONS	32
6.1 Schedule of Evaluations (SOE)	32
6.2 Timing of Evaluations	34
6.3 Instructions for Evaluations	36
7.0 ADVERSE EVENTS AND STUDY MONITORING	42
7.1 Definition of Adverse Events	42
7.2 Adverse Event Collection Requirements	42
7.3 Expedited Adverse Event (EAE) Reporting to DAIDS	43
7.4 Study Monitoring	44
8.0 CLINICAL MANAGEMENT ISSUES	45
8.1 Toxicity	45
8.2 Specific Management of Toxicities Related to Study-Provided Drugs	46

CONTENTS (Cont'd)

Page

9.0	CRITERIA FOR DISCONTINUATION	48
9.1	Premature Study Treatment Discontinuation	48
9.2	Premature Study Discontinuation	48
10.0	STATISTICAL CONSIDERATIONS	48
10.1	General Design Issues	48
10.2	Outcome Measures	49
10.3	Randomization and Stratification	49
10.4	Sample Size and Accrual	50
10.5	Data and Safety Monitoring	50
10.6	Analyses	50
11.0	PHARMACOLOGY PLAN	52
11.1	Pharmacology Objectives	52
11.2	Pharmacology Study Design	52
11.3	Primary and Secondary Data, Modeling, and Data Analysis	53
11.4	Anticipated Outcomes	53
12.0	DATA COLLECTION AND MONITORING	53
12.1	Records to Be Kept	53
12.2	Role of Data Management	53
12.3	Clinical Site Monitoring and Record Availability	54
13.0	PARTICIPANTS	54
13.1	Institutional Review Board (IRB) Review and Informed Consent	54
13.2	Participant Confidentiality	54
13.3	Study Discontinuation	54
14.0	PUBLICATION OF RESEARCH FINDINGS	55
15.0	BIOHAZARD CONTAINMENT	55
16.0	REFERENCES	56
	APPENDIX I: SAMPLE INFORMED CONSENT	60

SITES PARTICIPATING IN THE STUDY

A5366 is a multicenter study open only at US AIDS Clinical Trials Group (ACTG) clinical research sites (CRSs). Refer to the protocol webpage on the ACTG member website for the list of eligible sites.

PROTOCOL TEAM ROSTER (Cont'd)

PROTOCOL TEAM ROSTER

Co-Chairs

Rajesh Gandhi, MD
Massachusetts General Hospital (MGH)
CRS
Cox 5th Floor
55 Fruit Street
Boston, MA 02114
Phone: 617-724-9690
Fax: 617-726-7653
E-mail: rgandhi@partners.org

Eileen Scully, MD, PhD
Johns Hopkins University School of
Medicine
Pre-Clinical Teaching Building, Suite 211
725 N Wolfe Street
Baltimore, MD 21205
Phone: 203-906-6071
E-mail: escully1@jhmi.edu

Vice Chair

Daniel Kuritzkes, MD
Brigham and Women's Hospital
Therapeutics (BWHT) CRS
Section of Retroviral Therapeutics
Harvard Medical School
65 Landsdowne Street, Room 447
Cambridge, MA 02139
Phone: 617-768-8371
Fax: 617-768-8738
E-mail: dkuritzkes@partners.org

DAIDS Clinical Representative

Catherine Godfrey, MD
HIV Research Branch
TRP, DAIDS, NIAID, NIH
Room 9E42
5601 Fishers Lane
Rockville, MD 20852
Phone: 240-627-3074
Fax: 301-432-9282
E-mail: cgodfrey@niaid.nih.gov

Clinical Trials Specialist

Lara Hosey, MA
ACTG Network Coordinating Center
Social & Scientific Systems, Inc.
8757 Georgia Avenue, 12th Floor
Silver Spring, MD 20910
Phone: 301-628-3395
Fax: 301-628-3302
E-mail: LHosey@s-3.com

Statisticians

Evgenia Aga, MS
Statistical and Data Analysis Center
Harvard TH Chan School of Public Health
FXB Building, Room 504
651 Huntington Avenue
Boston, MA 02115
Phone: 617-432-3029
Fax: 617-432-3163
E-mail: eaga@sdac.harvard.edu

Ronald Bosch, PhD
Statistical and Data Analysis Center
Harvard TH Chan School of Public Health
FXB Building, Room 603
651 Huntington Avenue
Boston, MA 02115-6017
Phone: 617-432-3024
Fax: 617-432-2843
E-mail: ronbosch@sdac.harvard.edu

Data Manager

Apsara Nair, MSc
Frontier Science & Technology Research
Foundation, Inc.
4033 Maple Road
Amherst, NY 14226
Phone: 716-834-0900 Ext. 7293
Fax: 716-834-8432
E-mail: nair@fstrf.org

PROTOCOL TEAM ROSTER (Cont'd)

DAIDS Pharmacist

Irene Rwakazina, PharmD
Pharmaceutical Affairs Branch
DAIDS, OCSO, PAB
5601 Fishers Lane
Room 9E14
Rockville, MD 20892
Phone: 301-761-7269
E-mail: irene.rwakazina@nih.gov

Immunologist

Scott Sieg, PhD
Case CRS
Wood Building, Room W-119
2109 Adelbert Road
Cleveland, OH 44106-4984
Phone: 216-368-1965
Fax: 216-368-1969
E-mail: sfs2@case.edu

Virologist

Athe Tsibris, MD
Harvard University VSL
65 Landsdowne Street, Room 422
Cambridge, MA 02139
Phone: 617-417-1162
Fax: 617-768-8738
E-mail: atsibris@partners.org

Pharmacologists

Qing Ma, PhD
University of Rochester Adult HIV
Therapeutic Strategies Network CRS
Pharmacy Practice
315 Kapoor Hall
Buffalo, NY 14214
Phone: 716-881-7500
Fax: 716-849-6890
E-mail: qingma@buffalo.edu

Pharmacologists (Cont'd)

Gene Morse, PharmD
University of Rochester Adult HIV
Therapeutic Strategies CRS
Translational Pharmacology Research Core
NYS Center of Excellence in Bioinformatics
and Life Sciences
701 Ellicott Street
Buffalo, NY 14203
Phone: 716-881-7464
E-mail: emorse@buffalo.edu

Investigators

Nancie Archin, PhD
Chapel Hill CRS
120 Mason Farm Road, CB 7042
Genetic Medicine Building, Room 2049H
Chapel Hill, NC 27599-7042
Phone: 919-962-0180
E-mail: nancie_archin@med.unc.edu

Nicolas Chomont, PhD
Université de Montréal
Centre de Recherche du CHUM
900, rue St-Denis, Tour Viger, R09.430
Montreal, Quebec H2X 0A9
CANADA
Phone: 1-514-890-8000 Ext. 31266
E-mail: nicolas.chomont@umontreal.ca

Elizabeth Connick, MD
University of Arizona CRS
1501 N. Campbell Ave
P.O. Box 245039
Tucson, AZ 85724
Phone: 520-626-6887
Fax: 520-626-5183
E-mail: connicke@email.arizona.edu

PROTOCOL TEAM ROSTER (Cont'd)

Investigators (Cont'd)

Steven Deeks, MD
University of California, San Francisco
HIV/AIDS CRS
Building 80, Ward 84
Box 0874
995 Potrero Avenue
San Francisco, CA 94110-2859
Phone: 415-476-4082 Ext. 404
Fax: 415-476-6953
E-mail: steven.deeks@ucsf.edu

Monica Gandhi, MD, MPH
University of California, San Francisco
HIV/AIDS CRS
4th floor, Building 80, Room 423D
995 Potrero Avenue
San Francisco, CA 94110
Phone: 415-476-4082 x 127
Fax: 415-476-6953
E-mail: monica.gandhi@ucsf.edu

Jonathan Karn, PhD
Case Western Reserve University
Department of Molecular Biology &
Microbiology
10900 Euclid Avenue, LC 4960
Cleveland, OH 44106
Phone: 216-368-3915
Fax: 216-368-3055
E-mail: jxk153@case.edu

Field Representative

Erin Hoffman, BS
Chapel Hill CRS
130 Mason Farm Road
Chapel Hill, NC 27599
Phone: 919-843-0720
Fax: 919-966-8928
E-mail: erin_stephenson@med.unc.edu

Laboratory Technologist

Francoise Giguel, BA
Massachusetts General Hospital
Partners AIDS Research Center
Division of Infectious Diseases
65 Landsdowne Street
Room 435
Cambridge, MA 02139
Phone: 617-768-8374
Fax: 617-768-8299
E-mail: fgiguel@mgh.harvard.edu

Community Scientific Subcommittee (CSS)Representative

Kate Borloglou, BS
Ohio State University CRS
5789 Redsand Road
Hilliard, OH 43026
Phone: 614-353-2944
E-mail: borloglou@gmail.com

Industry Representative

Kathleen Squires, MD
Merck Research Labs
351 N. Sumneytown Pike,
Mailstop UG3CD-28
Upper Gwynned, PA 19454
Phone: 267-305-6779
E-mail: kathleen.squires@merck.com

Laboratory Data Managers

Andrew Bakatsias, BS
Frontier Science & Technology Research
Foundation, Inc.
4033 Maple Road
Amherst, NY 14226
Phone: 716-834-0900 Ext. 7201
E-mail: bakatsia@fstfrf.org

Alex Benns, BS
Frontier Science & Technology Research
Foundation, Inc.
4033 Maple Road
Amherst, NY 14226
Phone: 716-898-7464
E-mail: benns.alex@fstfrf.org

STUDY MANAGEMENT

All general questions concerning this protocol should be sent to actg.teamA5366@fstrf.org via e-mail. The appropriate team member will respond with a "cc" to actg.teamA5366@fstrf.org. A response should generally be received within 24 hours (Monday through Friday).

Protocol E-mail Group

Sites should contact the User Support Group at the Data Management Center (DMC) as soon as possible to have the relevant personnel at the site added to the [actg.protA5366](mailto:actg.protA5366@fstrf.org) e-mail group. Include the protocol number in the e-mail subject line.

- Send an e-mail message to actg.user.support@fstrf.org.

Clinical Management:

For questions concerning entry criteria, toxicity management, concomitant medications, and co-enrollment, contact the core protocol team.

- Send an e-mail message to actg.corea5366@fstrf.org. Include the protocol number, patient identification number (PID), and a brief relevant history.

Laboratory

For questions specifically related to virologic laboratory tests, contact the Protocol Virologist:

- Send an e-mail message to actg.teamA5366@fstrf.org (ATTENTION: Athe Tsibris).

For questions specifically related to pharmacologic laboratory tests, contact the Protocol Pharmacologists:

- Send an e-mail message to actg.teamA5366@fstrf.org (ATTENTION: Qing Ma and Gene Morse).

For questions specifically related to immunologic laboratory tests, contact the Protocol Immunologist:

- Send an e-mail message to actg.teamA5366@fstrf.org (ATTENTION: Scott Sieg).

Data Management

- For nonclinical questions about transfers, inclusion/exclusion criteria, electronic case report forms (eCRFs), randomization/registration, and other data management issues, contact the Data Manager. Completion guidelines for eCRFs and participant-completed CRFs can be downloaded from the FSTRF website at www.frontierscience.org.
- For transfers, reference the Study Participant Transfer SOP 119, and contact Apsara Nair directly.
- For other questions, send an e-mail message to actg.teamA5366@fstrf.org (ATTENTION: Apsara Nair).
- Include the protocol number, PID, and a detailed question.

STUDY MANAGEMENT (Cont'd)

Randomization

For randomization questions or problems and study identification number (SID) lists:

- Send an e-mail message to rando.support@fstrf.org or call the DMC Randomization Desk at 716-834-0900, extension 7301.

DMC Portal & Medidata Rave Problems

Contact DMC User Support

- Send an e-mail message to actg.user.support@fstrf.org or call 716-834-0900 x7302.

Protocol Document Questions

For questions concerning the protocol document, contact the Clinical Trials Specialist.

- Send an e-mail message to actg.teamA5366@fstrf.org (ATTENTION: Lara Hosey).

Copies of the Protocol

- To request a hard copy of the protocol, send an e-mail message to ACTGNCC@s-3.com (ATTENTION: Diane Delgado).
- Electronic copies can be downloaded from the ACTG website at <https://www.actgnetwork.org>.

Product Package Inserts and/or Investigator Brochures

To request copies of product package inserts or investigator brochures, contact the DAIDS Regulatory Support Center (RSC) at RIC@tech-res.com or call 301-897-1708.

Protocol Registration

For protocol registration questions, send an e-mail message to Protocol@tech-res.com or call 301-897-1707.

Protocol Activation

For questions related to protocol activation, contact the Clinical Trials Specialist or ACTG Site Coordination Group at actgsitecoordination@s-3.com.

Study Product

For questions or problems regarding study product, dose, supplies, records, and returns, call Irene Rwakazina, Protocol Pharmacist, at 301-761-7269.

Study Drug Orders

Call the Clinical Research Products Management Center (CRPMC) at 301-294-0741.

Expedited Adverse Event (EAE) Reporting/Questions

Contact DAIDS through the RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com or call 1-800-537-9979 or 301-897-1709; or fax 1-800-275-7619 or 301-897-1710.

STUDY MANAGEMENT (Cont'd)

Telephone Calls

Sites are responsible for documenting telephone calls made to A5366 team members.

- Send an e-mail message to actg.teamA5366@fstrf.org.

Protocol-Specific Web Page

Additional information about management of the protocol can be found on the protocol-specific web page (PSWP).

GLOSSARY OF PROTOCOL-SPECIFIC TERMS

CA-RNA	cell associated HIV-1 RNA
ESR-1	estrogen receptor-1
HDACi	histone deacetylase inhibitor
SERM	selective estrogen receptor modulator
shRNA	small hairpin RNA
TCR	T cell receptor

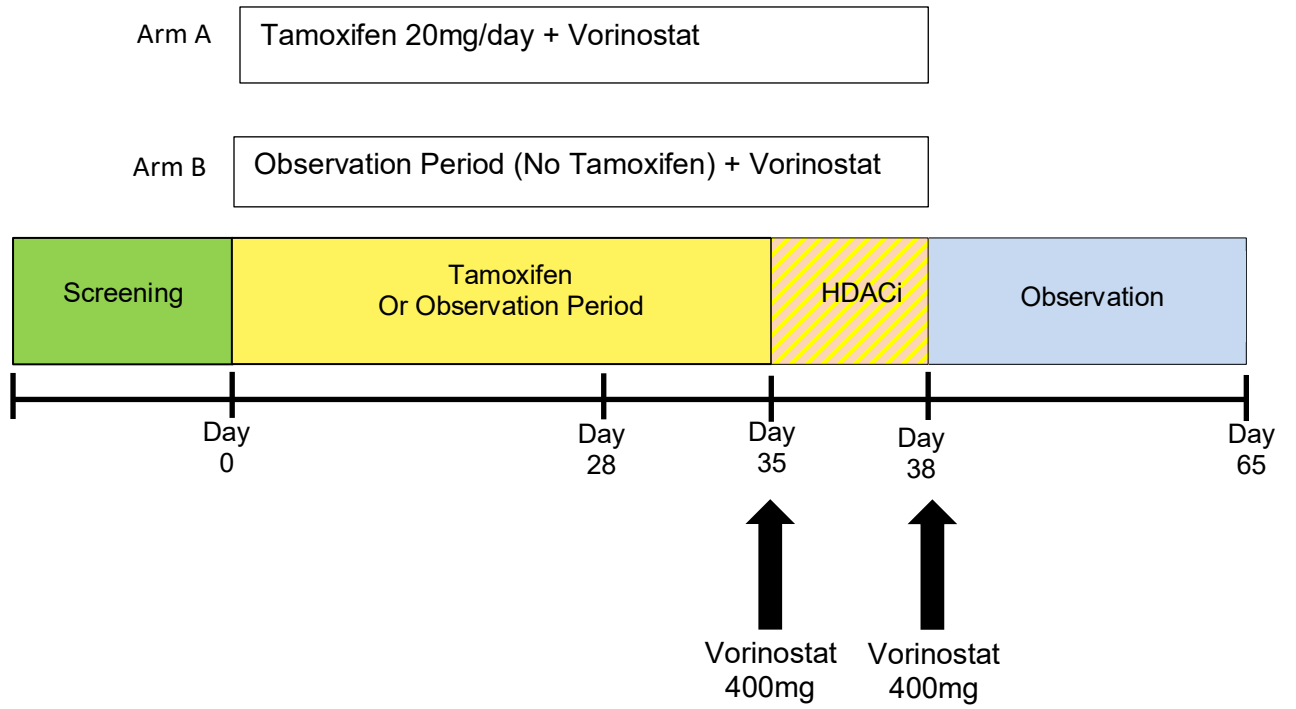
SCHEMA

A5366

Selective Estrogen Receptor Modulators to Enhance the Efficacy of Viral Reactivation with
Histone Deacetylase Inhibitors

<u>DESIGN</u>	Randomized, open-label, exploratory study assessing the effects of tamoxifen exposure in combination with vorinostat compared to vorinostat alone on viral reactivation among HIV-1 infected post-menopausal women with virologic suppression on antiretroviral therapy (ART).
<u>DURATION</u>	65 days per participant
<u>SAMPLE SIZE</u>	30 participants (20 participants in Arm A, 10 participants in Arm B)
<u>POPULATION</u>	HIV-1-infected post-menopausal women (age 18-65) with virologic suppression on ART
<u>REGIMENS</u>	<u>Arm A:</u> Days 0-38: Tamoxifen 20 mg orally (PO) once a day (QD) Day 35: Vorinostat 400 mg PO x 1 Day 38: Vorinostat 400 mg PO x 1 Day 39-65: Observation <u>Arm B:</u> Days 0-38: Observation period (no tamoxifen) Day 35: Vorinostat 400 mg PO x 1 Day 38: Vorinostat 400 mg PO x 1 Day 39-65: Observation

Figure 1: Study Design



1.0 HYPOTHESIS AND STUDY OBJECTIVES

1.1 Hypothesis

Treatment with the selective estrogen receptor modulator (SERM) tamoxifen will enhance the ability of the histone deacetylase inhibitor (HDACi) vorinostat to reverse HIV-1 latency. In HIV-1-infected participants on antiretroviral therapy (ART), combination therapy with tamoxifen and vorinostat will lead to higher levels of HIV-1 cell-associated RNA than will vorinostat alone.

1.2 Primary Objectives

- 1.2.1 Determine the safety and tolerability of combined therapy with the SERM tamoxifen and the HDACi vorinostat among HIV-infected post-menopausal women on ART.
- 1.2.2 Evaluate the level of HIV-1 reactivation following treatment with vorinostat versus a combination of tamoxifen and vorinostat as measured by cell associated HIV-1 RNA (CA-RNA) in CD4+ T cells (primary efficacy endpoint) among HIV-infected post-menopausal women on ART.

1.3. Secondary Objectives

- 1.3.1 Evaluate the impact of therapy with vorinostat versus a combination of tamoxifen and vorinostat on residual viremia, as measured by a single copy assay.
- 1.3.2 Assess the impact of therapy with vorinostat versus a combination of tamoxifen and vorinostat on markers of HIV-1 persistence, as measured by total DNA levels.

1.4. Exploratory Objectives

- 1.4.1 Assess the impact of therapy with vorinostat versus a combination of tamoxifen and vorinostat on markers of HIV-1 persistence, including:
 - 1.4.1.1 Integrated HIV-1 DNA levels
 - 1.4.1.2 HIV p24 antigen expression by ultrasensitive assay
 - 1.4.1.3 Ex vivo HIV-1 RNA expression assays
- 1.4.2 Measure levels of HIV-specific immunity and activation before and after therapy with vorinostat versus a combination of tamoxifen and vorinostat, including:
 - 1.4.2.1 HIV-1-specific immune responses
 - 1.4.2.2 T cell activation
 - 1.4.2.3 Expression of exhaustion markers on T cells
- 1.4.3 Assess the effect of vorinostat on bulk levels of histone acetylation.

- 1.4.4 Assess pharmacokinetic interactions between vorinostat and tamoxifen.
- 1.4.5 Assess pharmacodynamic interactions between vorinostat and tamoxifen, as measured by the relationship between drug levels and the degree of histone acetylation and HIV-1 expression.
- 1.4.6 Assess innate cellular markers and soluble factors in relationship to treatment intervention arms or virologic outcome measures.

2.0 INTRODUCTION

2.1 Background

A critical barrier to HIV-1 eradication is the maintenance of transcriptionally silent, replication competent proviruses in resting CD4+ T cells [1-3]. These proviruses, which cannot be detected by the immune system, comprise a reservoir with a very slow rate of decay [4,5] from which viremia can rebound when antiretroviral therapy is withdrawn. One pathway to eradication of HIV-1 requires activation of proviral transcription coupled with immune surveillance and elimination of residual cells harboring virus [6]. The potential of this approach has been highlighted by recent work delineating some of the mechanisms whereby HIV-1 establishes and maintains latency.

Controls on HIV-1 transcription include cellular signaling machinery and epigenetic silencing modifications, with recent work highlighting several potential pathways for therapeutic interventions to release transcriptional control [7]. Clinical trials have tested candidate latency reversal agents, with demonstrated increases in measures of HIV-1 RNA after treatment of study participants with the histone deacetylase inhibitors (HDACi) vorinostat [8,9], panobinostat [10], romidepsin [11,12], and the phosphatase and tensin homolog inhibitor, disulfiram [13]. However, despite the induction of HIV-1 RNA production, none of these agents has led to a statistically significant change in the size of the HIV reservoir. Further work is needed to optimize both latency reversal and subsequent immune clearance of the activated reservoir.

Another feature of early results from HIV cure studies has been the substantial heterogeneity in the level of responses to reactivation agents. The sources of interindividual variability in response are likely multiple, but a clearer understanding of these factors will help target specific interventions to the populations most likely to benefit. One potential source of variation is the sex of the individual. Numerous studies have demonstrated substantial sex-based differences between men and women in terms of vaccine responses, autoimmunity, and the acquisition and progression of infectious diseases [14,15]. Women bear a significant burden of the global HIV epidemic, estimated at slightly more than half of worldwide infections, with a marked risk for young women to acquire HIV in sub-Saharan Africa (UNAIDS Global Factsheet, 2017). Despite this, women are underrepresented in clinical trials relevant to HIV cure [16]. As a

relevant cure strategy will need to have efficacy for both men and women, studies addressing key biological differences will be critical to either identify significant distinctions in cure strategies by sex or to rationally design cure studies to be inclusive of women.

With this clinical trial, we seek to investigate a pathway to enhance viral reactivation in vivo in women, with potential implications for cure efforts in both men and women.

2.2 Rationale

A5366 is designed primarily to evaluate the safety of 38 days of tamoxifen therapy followed by two doses of vorinostat with continued tamoxifen and the effectiveness of this combination on latent virus reactivation in women who are suppressed on ART in comparison to the effects of vorinostat alone.

Rationale for study treatment regimen

One notable barrier to efforts at eradication lies in the inefficiency of current methods of latency reversal. Even after maximal activation of T cells in vitro, a relatively small proportion of proviruses is transcribed, and notably a significant proportion of the noninduced proviruses retain intact genomes [17]. Agents investigated in clinical trials to date have significantly lower efficiency at HIV-1 induction in vivo compared to the maximal induction observed in vitro with T cell receptor stimulation, requiring further research into potential synergistic approaches to enhance reactivation with a combination of agents. In an unbiased, genome-wide small hairpin RNA (shRNA) screen, Karn et al identified the estrogen receptor-1 (ESR-1) as a cellular factor critical to HIV-1 latency reversal in the Jurkat cell model (Figure 2.2-1).

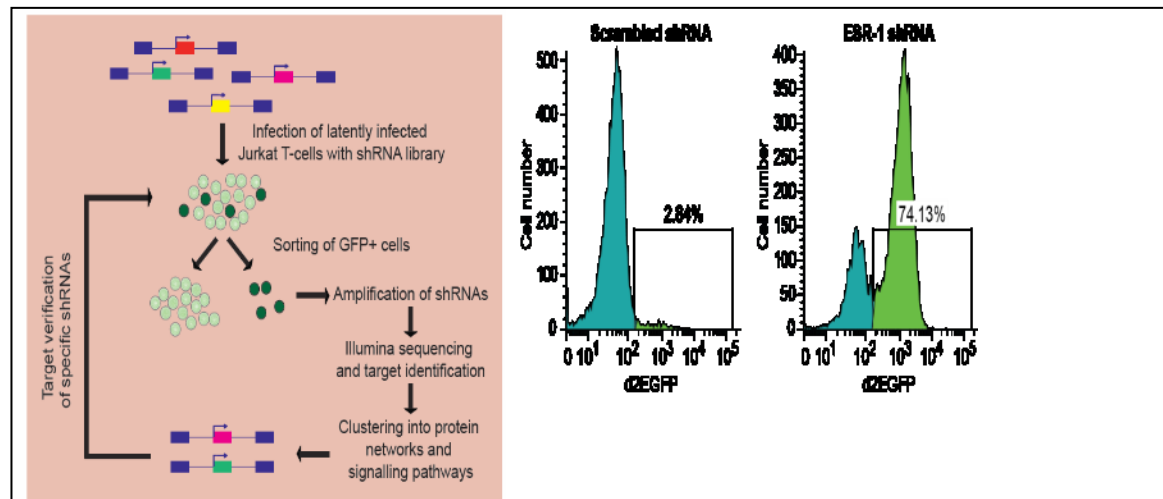


Figure 2.2-1. shRNA screen strategy that identified the ESR-1 as a critical cellular factor in latency reversal (left panel). Silencing of the ESR-1 with a shRNA activates transcription of HIV-1 as indicated by eGFP expression whereas a scrambled shRNA shows no activation.

These findings were validated in a primary cell model of HIV-1 latency [18], where Karn and colleagues demonstrated that exposure to estrogen (or stilbestrol, an estrogen agonist) decreased HIV-1 expression as assessed by Nef expression measured by flow cytometry. In contrast, treatment with an estrogen antagonist (Fulvestrant or gossypol) led to increased HIV-1 expression in combination with other activation stimuli (Figure 2.2-2).

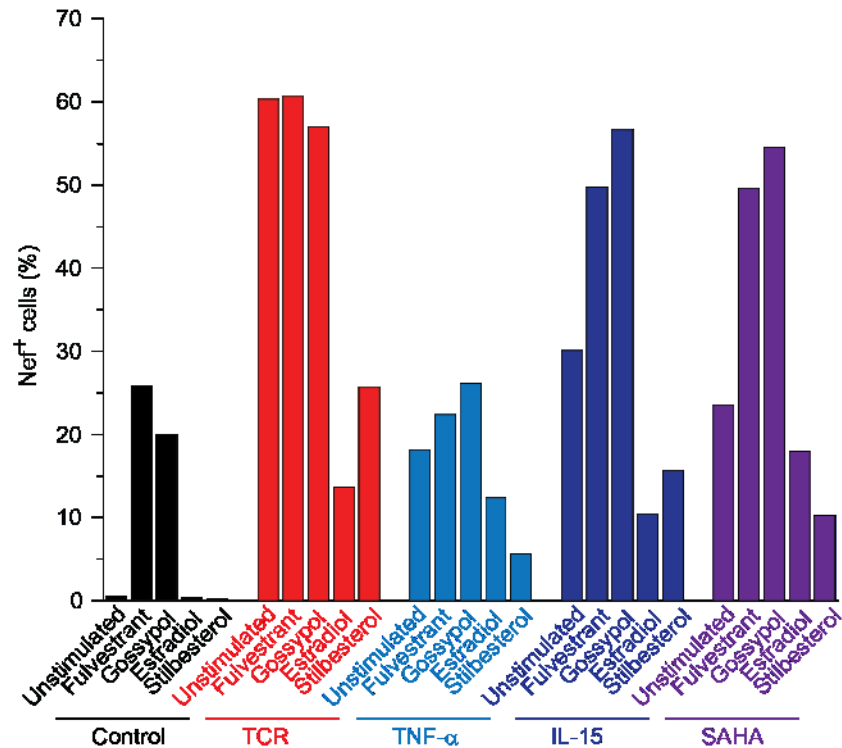


Figure 2.2-2. Synergistic reactivation of HIV transcription in primary T-cells by ESR-1 antagonists and **vorinostat** or IL-15. HIV transcription was assessed by flow cytometry using an antibody to HIV-Nef. β -estradiol and stilbestrol are able to potently block reactivation under all conditions including TCR activation.

Taken together, these data suggest that the estrogen receptor is a key cellular factor in modulating HIV-1 latency reversal, and, thereby, a potential therapeutic target. To probe the physiologic relevance of these observations, a cohort of HIV-infected women and men (matched on age, duration of viral suppression, CD4 cell nadir and current CD4 cell count) with full viral suppression on ART was recruited for assessment of multiple metrics of HIV persistence. The women in this study had full viral suppression of at least a year, had active menstrual cycles, and were not on hormonal contraception or replacement. Leukapheresis samples from women in this cohort on fully suppressive ART were exposed in vitro to T cell receptor (TCR) stimulation, or latency reversal treatments, including IL-15 or vorinostat in combination with either estradiol or an

estrogen receptor antagonist, and HIV-1 RNA induction was subsequently assessed. Strikingly, estradiol potently inhibited activation of HIV-1 transcription, including TCR-induced reactivation. Conversely, incubation with an estrogen receptor antagonist (ICI) enhanced the activity of vorinostat (Figure 2.2-3).

The effect of combining TCR stimulation with estradiol ex vivo was significantly influenced by biologic sex; leukapheresis samples from men displayed a 43-fold higher induction of HIV RNA with exposure to TCR+estradiol compared to samples from women ($p < 0.01$ by t-test). Conversely, the combination of vorinostat and the estrogen antagonist (ICI) had a larger induction effect among samples from women compared to men (fold effect 1.7, $p < 0.01$). These ex vivo results are consistent with the cell line and primary cell in vitro models summarized above [14,15], suggesting an important role for hormonal modulation of HIV-1 activity with a likely sensitivity to these hormonal pathways among female participants.

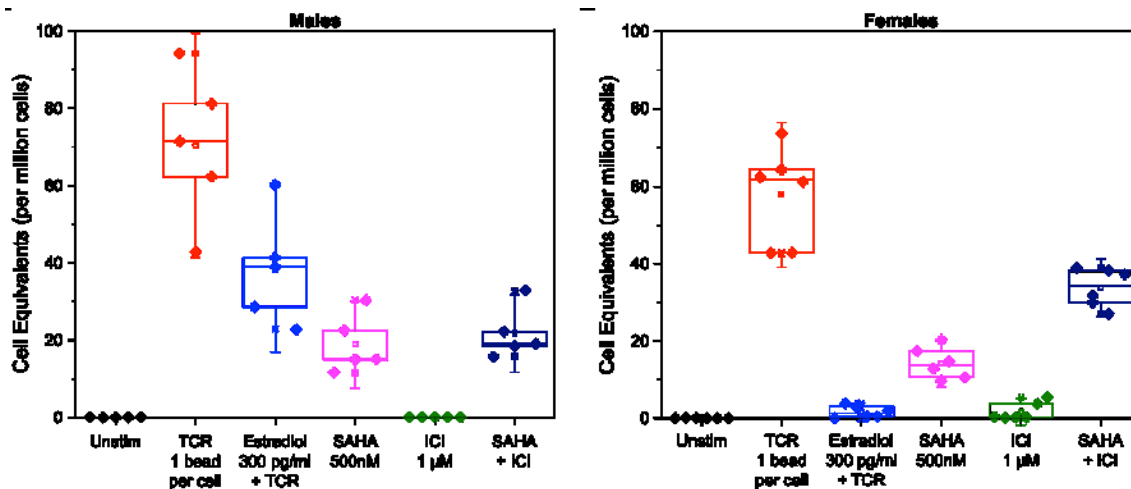


Figure 2.2-3. Ex vivo induction of HIV-1 transcription in leukapheresis samples from ART-treated women. TCR stimulated reactivation is abrogated in the presence of estradiol. Conversely, treatment with the estrogen antagonist ICI **in combination** with vorinostat enhanced the transcription to higher levels than seen with vorinostat alone. This effect is more pronounced in women than in men.

These results have critical implications for eradication studies. First, they suggest that concurrent exposure to estrogen may limit the efficacy of latency reversal agents currently under study. Given the small size and exploratory nature of clinical trials of cure to date, the stark underrepresentation of women in trials relevant to cure [16], and variation of endogenous estrogen levels over the course of a menstrual cycle, these findings argue for boosting the inclusion of women in trials with careful investigation of their hormonal milieu. Further, these results suggest a novel biological pathway as a target for synergistic activation of HIV-1 transcription in combination with other latency reversal classes, such as HDACi. While in vitro and ex vivo studies offer significant support for the role of hormones in latency reversal, the complexity of the system in vivo

obligates further investigation. With these data, we propose to study the effects of in vivo combined exposure to selective estrogen receptor modulators and latency reversal agents via an exploratory clinical trial.

Rationale for selection of study drugs

SERMs are a well-characterized class of drugs with indications in the treatment of and chemoprophylaxis for breast cancer and postmenopausal osteoporosis. The effects of SERMs are tissue specific, and can include both agonist and antagonist activity, depending on the agent. These drugs have generally favorable safety profiles, with risks notable for venous thromboembolism, cerebrovascular events, and inducing menopausal symptoms [19]. Tamoxifen is a SERM with an extensive record of clinical use with favorable pharmacologic properties. Tamoxifen is taken orally, has a terminal half-life of 5 to 7 days, and reaches steady state concentrations after 4 weeks of dosing. In vitro testing directly supports the synergistic activity of tamoxifen in combination with vorinostat on transcription as shown in Figure 2.2-4, with this effect being more pronounced in females (representative in the right panel) versus males (left panel). Finally, the combination of the HDACi vorinostat with tamoxifen has already been tested among patients in a Phase II trial with refractory breast cancer and was well-tolerated [20].

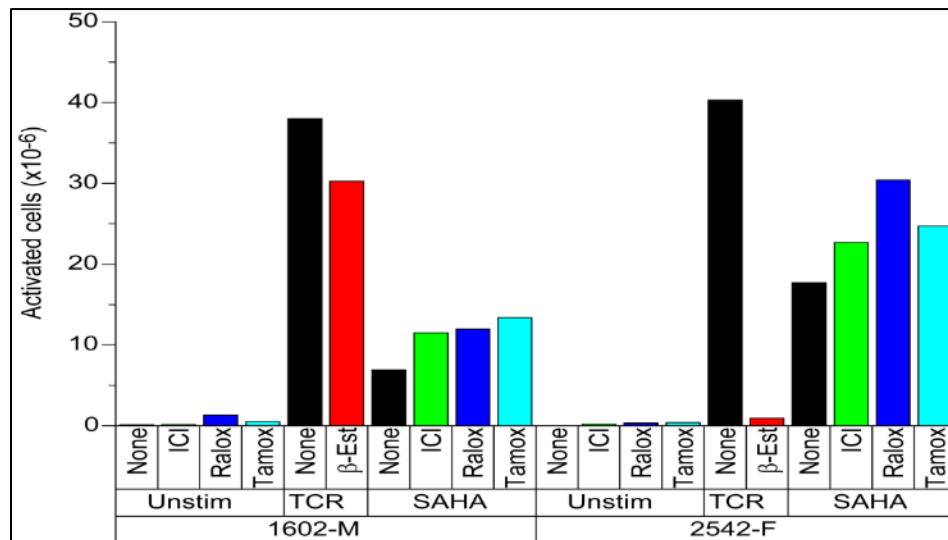


Figure 2.2-4. Direct assessment of the synergy between tamoxifen and vorinostat confirms that in a female participant there is synergistic activation of transcription as measured by the EDITS assay described above.

As above, multiple agents are currently under investigation for potential efficacy in latency reversal. Of those that have been used in clinical trials in the United States and internationally, vorinostat, panobinostat, and romidepsin have all shown some efficacy in induction of HIV RNA transcription. Panobinostat has the caveat of concerns about potential cardiac toxicity requiring more intensive monitoring during the period of administration. Romidepsin has anti-estrogenic activity, competing with β -estradiol for

binding to estrogen receptors in vitro and showing reversible effects on testes and ovaries in mice [21]. To isolate the effects of tamoxifen-mediated estrogen blockade, we have opted to use vorinostat, with the safety of this combination already demonstrated in the context of breast cancer.

Taken together, preliminary data suggest a role for estrogen in the control of HIV-1 transcription in cell line models, primary cell latency models and in ex vivo models using leukapheresed patient samples. In vitro assays indicate that the combination of HDACi and SERMs enhances HIV transcription. Vorinostat and tamoxifen have demonstrated safety in combination in other clinical contexts. We therefore propose to evaluate the impact of this combination on HIV-1 transcription in vivo to hopefully define a novel pathway to enhance HIV-1 reactivation.

Rationale for pharmacologic studies

Tamoxifen is a SERM that is widely used for the treatment of estrogen receptor- α -positive breast cancer. The formation of tamoxifen active metabolites, including N-desmethyl-tamoxifen, 4-hydroxy-tamoxifen, and 4-hydroxy-N-desmethyltamoxifen (endoxifen), is predominantly catalyzed by CYP3A4/5 and CYP2D6 [22]. Thus, alterations of tamoxifen metabolism through a modulation of the CYP system may directly affect the efficacy and toxicity of tamoxifen. Inhibition of the CYP3A4 isoform with protease inhibitors, i.e., darunavir/ritonavir or cobicistat, would potentially decrease drug activity due to a significant decrease of active metabolite formation. The impact of CYP3A4 and -2D6 induction by efavirenz, however, is difficult to predict in that increased generation of active metabolites could result in both improved efficacy and/or worse adverse effects, whereas a reduction in tamoxifen efficacy might be also possible due to overall increased systemic clearance [23]. There are no data to confirm these theoretical drug interactions between tamoxifen and antiretrovirals. A significant reduction in tamoxifen (86%) and N-desmethyl-tamoxifen (38%) exposures by rifampicin has been reported in a randomized, placebo-controlled, crossover study, suggesting that the efficacy of tamoxifen could be compromised by concurrent use of CYP3A4 inducers [24]. Members of the non-nucleoside reverse transcriptase inhibitor class (NNRTIs), such as efavirenz and rifampin, are CYP3A4 inducers. However, the clinical significance of interactions between tamoxifen and efavirenz remains unclear as efavirenz exhibits significantly weaker inductive effects (~3 fold) in comparison to that of rifampin (~6 fold) [25].

The modulating effects of CYP system by tamoxifen have been reported with conflicting results from studies using in vitro cell systems and animal models where both induction and inhibition of CYP3A4 have been noted [26-28]. The current consensus is that tamoxifen exhibits mild CYP3A4 inductive effects based on findings from a case report and a clinical study with unexpected 38% lower letrozole concentrations following 6-week combination therapy with tamoxifen [29,30]. There are no data currently available that document the effects of tamoxifen-mediated CYP3A4 induction on antiretrovirals. A recommendation on the use of alternative non-CYP3A4 dependent regimens was made based on the theoretical interaction that tamoxifen might decrease protease inhibitor and elvitegravir concentrations. This recommendation, however, does not consider the potent CYP3A4 inhibition by the pharmacoenhancers (ritonavir and cobicistat) used in

combination with protease inhibitors and elvitegravir [31]. While a significant interaction between tamoxifen and darunavir/ritonavir or elvitegravir/cobicistat is unlikely, this requires confirmation by drug interaction studies. Tamoxifen is also an inhibitor of P-glycoprotein (P-gp) and endoxifen is a substrate of P-gp [32]. No P-gp mediated tamoxifen drug interactions, however, have been reported to date.

Vorinostat exhibits nonlinear pharmacokinetics in Phase I studies [33,34]. C_{max} increased with dose between 100-400 mg, but not when the dose exceeded 400 mg. Its mean t_{1/2} ranged from ~2 hour (400 mg daily) to 4 hour (500 mg daily) and Cl/F decreased from 605 (100 mg BID) to 313 l/hour (400 mg daily). Meals containing high fat increase vorinostat exposure by 38% and significantly delay the absorption rate [35]. Vorinostat is primarily metabolized through glucuronidation mediated by UGTs including -1A1, -1A3, -1A7, -1A8, -1A9, -1A10, -2B7, and -2B17 [36] into inactive metabolites, O-glucuronide and 4-anilino-4-oxobutanoic acid, whereas the cytochrome P450 system plays a negligible role in its metabolism [37], suggesting a low drug interaction potential between vorinostat and antiretrovirals. However, the inductive effects of vorinostat on multiple transporters including P-gp, breast cancer resistance protein, and multidrug resistance-associated proteins have been demonstrated in the in vitro studies, suggesting potential drug interactions with P-gp substrates and/or inhibitors [38,39].

Rationale for selection of study participants

This protocol focuses exclusively on women, seeking to specifically interrogate the role of the estrogen receptor pathway in HIV-1 latency reversal. Women represent approximately half of all individuals living with HIV-1 worldwide but are a minority of participants in clinical trials relevant to HIV-1 cure [16]. Clinical data suggests that there are relevant differences in virologic and immunologic measures between men and women and understanding how these differences may affect curative efforts is critical to ensure that an intervention will be likely to be efficacious in women as well as in men. For this initial trial, we have opted to include only postmenopausal women. The reasons for this are to maximize patient safety; due to concerns for potential genotoxicity of vorinostat, including only postmenopausal women eliminates concerns about future fertility. From the perspective of tamoxifen, the drug is less likely to induce menopausal symptoms in women who have already stopped menstruating and has a significant track record of use within this population. To verify the role of the estrogen receptor pathway in this population, we examined cryopreserved PBMCs from four postmenopausal women on fully suppressive ART followed as part of a longitudinal observational cohort (ACTG A5321). These samples were stimulated ex vivo and induction of HIV-1 RNA was measured using the EDITS (Envelope Detection by Induced Transcription-based Sequencing) assay, which measures inducible cell-associated HIV-1 RNA. The production of RNA is normalized to a calibration curve of known cell inputs with HIV RNA expression. These results demonstrated a strong susceptibility to estrogen-mediated repression of transcription as well as an increase in HIV-1 RNA production after a brief in vitro treatment with tamoxifen alone. In this set of samples, levels of HIV-1 RNA production induced by vorinostat were similar to levels induced by the combination of tamoxifen and vorinostat ([Figure 2.2-5](#)).

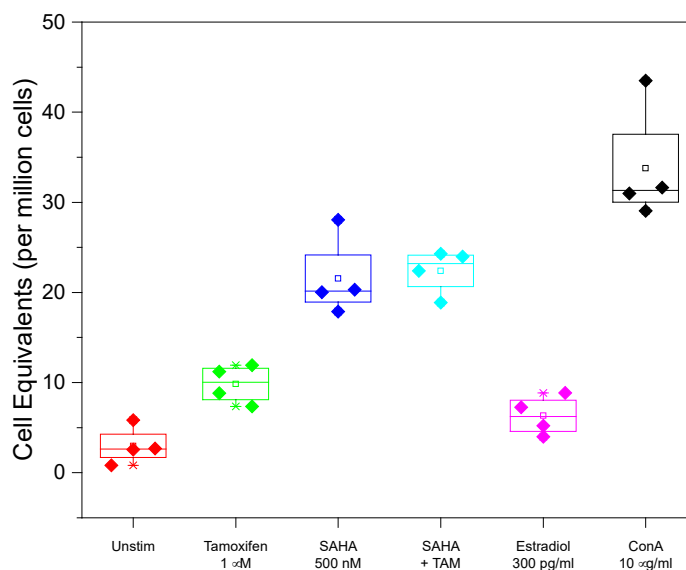


Figure 2.2-5. Induction of HIV-1 RNA as measured by the EDITS assay in samples from four postmenopausal women. Results demonstrate estrogen mediated suppression of T cell activation induced (ConA) HIV transcription and an increase in HIV-1 RNA production after exposure to tamoxifen.

Taken together these data support continued susceptibility to estrogen and estrogen antagonism in the postmenopausal population, with an antagonist inducing a measurable increase in transcription as a single agent. Whether there will be synergy between tamoxifen and vorinostat in vivo will depend on the hormonal milieu and effects of steady state estrogen antagonism. These factors are not fully modeled in vitro where the system is stripped of estrogen and there is only a brief exposure to antagonists. The proposed study will allow interrogation of the estrogen receptor pathway in vivo while minimizing risks to participants and will yield data on cure interventions for a critically understudied segment of the HIV-1 infected population.

3.0 STUDY DESIGN

This is a randomized, open-label, exploratory study among HIV-1-infected virologically suppressed postmenopausal women on ART to assess the effects of vorinostat versus tamoxifen and vorinostat on HIV-1 transcription. Participants (n=30) will be randomized 2:1 to receive tamoxifen 20 mg daily for 38 days (Arm A) plus two doses of vorinostat 400 mg (days 35 and 38) or to a 38-day observation period with no tamoxifen (Arm B) plus two doses of vorinostat 400 mg each (days 35 and 38).

The lead-in phase will allow tamoxifen to reach steady-state plasma levels and provides the opportunity to assess the influence of this SERM on HIV-1 activity and immune function alone prior to administration of vorinostat. Following HDACi treatment, all interventions will be discontinued and observation will continue until day 65.

4.0 SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

- 4.1.1 HIV-1 infection, documented by any licensed rapid HIV test or HIV enzyme or chemiluminescence immunoassay (E/CIA) test kit at any time prior to study entry and confirmed by a licensed Western blot or a second antibody test by a method other than the initial rapid HIV and/or E/CIA, or by HIV-1 antigen, plasma HIV-1 RNA viral load.

NOTE: The term “licensed” refers to a US FDA-approved kit. WHO (World Health Organization) and CDC (Centers for Disease Control and Prevention) guidelines mandate that confirmation of the initial test result must use a test that is different from the one used for the initial assessment. A reactive initial rapid test should be confirmed by either another type of rapid assay or an E/CIA that is based on a different antigen preparation and/or different test principle (e.g., indirect versus competitive), or a Western blot or a plasma HIV-1 RNA viral load.

- 4.1.2 Women between 18 and 65 years of age up until the 66th birthday at the time of study entry.
- 4.1.3 Women who are postmenopausal at the time of study entry and have agreed not to participate in assisted reproductive technology in the future. Menopausal status will be verified by any one of the following:
- 4.1.3.1 Age ≥ 40 years, amenorrhea ≥ 12 months, and FSH > 40 mIU/mL with a negative serum or urine β -HCG (urine test must have a sensitivity of ≤ 25 mIU/mL) and no oral or injectable exogenous hormone use within 12 months prior to study entry.
- 4.1.3.2 Any woman ≥ 18 years of age is eligible for consideration if there is a documented history of surgical removal of both of the ovaries > 6 months prior to study entry and no injectable or oral exogenous hormone therapy for a period of ≥ 12 months prior to study entry.
- 4.1.4 CD4+ cell count > 300 cells/uL obtained within 90 days prior to study entry at any US laboratory that has Clinical Laboratory Improvement Amendments (CLIA) certification or its equivalent.
- 4.1.5 Continuous ART containing nucleoside or nucleotide reverse transcriptase inhibitors with a non-nucleoside reverse transcriptase inhibitor, a pharmacologically-boosted protease inhibitor, or an integrase inhibitor for at least 2 years prior to enrollment with no known interruption in therapy for > 7 days within 90 days prior to study entry.

NOTE: Other ART regimens may be acceptable. Sites must consult the core protocol team for a case-by-case basis review of ART regimens not specified above.

NOTE: Regimens composed of three nucleoside reverse transcriptase inhibitors are not acceptable.

NOTE: Regimen changes within the 2-year period are acceptable, but candidates must have been on a stable regimen for at least 30 days prior to study entry.

- 4.1.6 At least one documented plasma HIV-1 RNA that is below the limit of detection of the FDA-approved assays (limit of detection: 75, 50, 40, or 20 copies/mL) between 13 and 24 months prior to the screening HIV-1 RNA in section 4.1.7.
- 4.1.7 Plasma HIV-1 RNA level of <20 copies/mL obtained by the Roche TaqMan v2.0 assay or <40 copies/mL obtained by the Abbott assay, within 90 days prior to study entry by any US laboratory that has CLIA certification or its equivalent.
- 4.1.8 The following laboratory values obtained within 21 days prior to study entry by any US laboratory that has CLIA certification or its equivalent.

- Absolute neutrophil count (ANC) ≥ 1500 neutrophils/mm³
- Hemoglobin ≥ 11.0 g/dL
- Platelet count $\geq 125,000$ platelets/uL
- Creatinine ≤ 1.3 x upper limit of normal (ULN) OR, if serum creatinine levels > 1.3 x ULN, calculated creatinine clearance (as estimated by the Cockcroft-Gault equation) ≥ 50 mL/min

NOTE: A calculator for the Cockcroft-Gault equation is available on the DMC website at www.fstrf.org.

- Aspartate aminotransferase (AST) (SGOT) ≤ 2 x ULN
- Alanine aminotransferase (ALT) (SGPT) ≤ 2 x ULN
- Alkaline phosphatase ≤ 2.5 x ULN
- Total bilirubin < 1.5 x ULN, OR if the total bilirubin is elevated, direct bilirubin will be measured and the potential participant is eligible if the direct bilirubin is < 2 x ULN

NOTE: For participants on atazanavir-based ART, if total bilirubin is > 1.5 x ULN and there is no transaminase elevation, enrollment is acceptable if the indirect bilirubin (calculated value of total bilirubin minus direct bilirubin) is < 3 x ULN.

- 4.1.9 No history of opportunistic infections within 90 days prior to study entry.
- 4.1.10 Karnofsky performance score ≥ 70 within 90 days prior to study entry.

4.1.11 Weight ≥ 52.2 kg at time of screening. (This stipulation is because of the blood draw volumes involved with this trial.)

4.1.12 Body Mass Index (BMI) ≤ 40 kg/m² at time of screening.

NOTE: A program for calculating BMI is available on the DMC website at www.fstrf.org.

4.1.13 HCV antibody negative result within 90 days prior to study entry or, for study candidates who are HCV antibody positive (based on testing performed at any time prior to study entry), a negative HCV RNA result obtained within 90 days prior to study entry.

4.1.14 Negative HBsAg result obtained within 90 days prior to study entry or a positive HBsAb result at any time prior to study entry.

4.1.15 QTc interval ≤ 450 milliseconds within 90 days prior to study entry.

NOTE: A program for calculating QTc by Fridericia's correction is available on the DMC website at www.fstrf.org.

4.1.16 Ability and willingness of potential participant to provide written informed consent.

4.2 Exclusion Criteria

4.2.1 History of venous thromboembolism.

4.2.2 History of stroke.

4.2.3 Known history of hypercoagulable state including Factor V Leiden mutation, Protein C and S deficiency, or decompensated cirrhosis.

4.2.4 Tobacco smoking or e-cigarette use within 90 days prior to study entry.

NOTE: If recent cessation of smoking, must have been without cigarettes and e-cigarettes for ≥ 90 days prior to study entry.

4.2.5 History of any malignancy requiring systemic chemotherapy or systemic immunotherapy.

4.2.6 History of endometrial or breast cancer or known genetic testing with BRCA positive results indicating an increased risk for breast and ovarian cancer.

NOTE: If additional genetic testing for breast or ovarian cancer exists, study sites should contact the core protocol team for review.

4.2.7 History of cardiac arrhythmia requiring surgical or ablative therapy.

4.2.8 History of myocardial infarction (MI) within 6 months prior to study entry, New York Heart Association (NYHA) class III or IV heart failure at any time prior to study entry, or personal or family history of prolonged QTc syndrome.

4.2.9 Use of immunomodulators (e.g., interleukins, interferons, cyclosporine), HIV vaccine, or investigational therapy within 60 days prior to study entry.

NOTE: Study candidates receiving stable physiologic glucocorticoid doses, defined as the equivalent of prednisone 10 mg/day or less, will not be excluded. Study candidates receiving inhaled or topical corticosteroids will not be excluded.

4.2.10 Any systemic hormonal therapy defined as oral or injectable contraceptives, estrogen and combined estrogen-progesterone replacement therapy in the prior 12 months, or a hormone containing IUD within 6 months prior to study entry.

NOTE: Hormonal therapy also includes aromatase inhibitors and suppressors of ovarian function including gonadotropin releasing hormone (GnRH) agonists and luteinizing-hormone releasing hormone (LH-RH) agonists. Topical estrogen cream use in the prior 12 months is acceptable, but should not be used during the study period.

4.2.11 Use of any study-prohibited medications that carry the risk of torsades de pointes within 60 days prior to study entry.

NOTE: A list of prohibited medications is available on the protocol-specific web page (PSWP).

4.2.12 Use of any study-prohibited medication in the HDACi class or use of any study-prohibited medication with HDACi-like activity within 60 days prior to study entry.

NOTE: A list of prohibited medications is available on the PSWP.

4.2.13 Known allergy/sensitivity or any hypersensitivity to components of study drugs or their formulations.

4.2.14 Active drug or alcohol use or dependence or psychiatric illness that, in the opinion of the site investigator, would interfere with adherence to study requirements.

4.2.15 Acute or serious illness requiring systemic treatment, antibiotics, and/or hospitalization within 90 days prior to study entry.

4.3 Study Enrollment Procedures

4.3.1 Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol consent forms approved, as appropriate, by their local institutional review board (IRB)/ethics

committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) WILL be reviewed and approved by the DAIDS PRO, and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approvals for an amendment, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all required documents have been received. Site-specific ICF(s) WILL NOT be reviewed by the DAIDS PRO. Sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

Once a candidate for study entry has been identified, details will be carefully discussed with the participant. The participant will be asked to read and sign the approved protocol consent form.

For participants from whom a signed informed consent has been obtained, an ACTG Screening Checklist must be entered through the DMC Participant Enrollment System.

4.3.2 Protocol Activation

Prior to enrollment, sites must complete the Protocol Activation Checklist found on the ACTG Member website. This checklist must be approved prior to any screening evaluations.

4.3.3 Randomization/Participant Registration

For participants from whom informed consent has been obtained, but who are deemed ineligible or who do not enroll into the initial protocol step, an ACTG Screening Failure Results form must be completed and keyed into the database. Participants who meet the enrollment criteria will be registered to the study according to standard ACTG DMC procedures.

4.4 Co-enrollment Guidelines

- Sites are encouraged to co-enroll participants in A5128, “Plan for Obtaining Informed Consent to Use Stored Human Biological Materials (HBM) for Currently Unspecified Analyses.” Co-enrollment in A5128 does not require permission from the A5366 protocol chairs. Due to blood volume restrictions, participants who elect to co-enroll in A5128 must defer A5128 blood draws until after all A5366 blood draws are completed.
- Sites are encouraged to co-enroll participants in A5332, “Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE).” Co-enrollment in A5332 does not require permission from the A5366 protocol chairs. A5332 participants are not permitted to enroll in A5366 until after they have completed their month 4 A5332 study visit.
- For specific questions and approval for co-enrollment in other studies, contact the core protocol team via e-mail as described in the Study Management section.

5.0 STUDY TREATMENT

Study treatment is defined as vorinostat and tamoxifen, both of which are provided by the study.

ART is required but will not be provided by the study.

5.1 Regimens

At entry, participants will be randomized (2:1) in open-label fashion to Arm A or Arm B:

	Days 0-38	Day 35	Day 38
Arm A	Tamoxifen 20 mg PO daily	Vorinostat 400 mg PO x 1	Vorinostat 400 mg PO x 1
Arm B	-----	Vorinostat 400 mg PO x 1	Vorinostat 400 mg PO x 1

5.2 Administration

Tamoxifen tablets should be swallowed whole, with water or another non-alcoholic drink. Tamoxifen can be taken with or without food. Tamoxifen should be taken consistently at approximately the same time of day, preferably in the morning. Participants in Arm A should complete all 38 days of tamoxifen. Sites should contact the core protocol team for guidance if participants have missed any doses of tamoxifen.

Vorinostat will be administered by clinic staff on days 35 and 38 in both Arms A and B along with a snack provided by the clinic. Vorinostat dosing must occur between 8:00 AM and 1:00 PM, local time. On day 35, all evaluations, including blood draws, must be done prior to administration of vorinostat. On day 38, the first blood draw for pharmacokinetic studies must be done prior to administration of vorinostat.

All vorinostat capsules (4 capsules) should be taken together as one dose on each day of administration. Vorinostat capsules should be swallowed whole, with water or another non-alcoholic drink. Vorinostat should be taken with food.

5.3 Study Product Formulation

Vorinostat is available as 100 mg capsules. The capsules are white, opaque hard gelatin capsules. The product should be stored at room temperature between 20–25°C (68–77°F), away from moisture and out of direct sunlight. Temperature excursions are permitted between 15–30°C (59–86°F). The capsules should not be opened or crushed. Caution should be exercised in handling vorinostat; the use of gloves is recommended. Direct contact of the powder in vorinostat capsules with the skin or mucous membranes should be avoided. If such contact occurs, wash thoroughly. The container should be kept tightly closed, retaining the silica gel desiccant in the bottle.

Tamoxifen is available as 20 mg tablets. The product should be stored at room temperature between 20–25°C (68–77°F). Dispense in a well-closed, light-resistant container.

5.4 Pharmacy: Product Supply, Distribution, and Accountability

5.4.1 Study Product Acquisition/Distribution

Vorinostat and tamoxifen will be made available to sites through the NIAID Clinical Research Products Management Center (CRPMC). Upon successful completion of protocol registration procedures, the products may be obtained by the site pharmacist by following instructions provided in the manual Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

5.4.2 Study Product Accountability

The site pharmacist is required to maintain complete records of all study products received from the NIAID CRPMC and subsequently dispensed. All study products must be stored in the pharmacy.

5.4.3 Final Disposition of Study Product

All unused study products remaining at US clinical research sites after the study is completed or terminated must be returned to the NIAID CRPMC (unless otherwise directed by the sponsor). Study products may also be returned to the CRPMC for other reasons, as requested by the sponsor. Site pharmacists will follow the relevant instructions for return of unused study products provided in the manual Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

5.5 Concomitant Medications

Whenever a concomitant medication or study agent is initiated or a dose changed, investigators must review the concomitant medication's and study agent's most recent package insert, Investigator's Brochure, or updated information from DAIDS to obtain the most current information on drug interactions, contraindications, and precautions. Additional drug information may be found on the ACTG Precautionary and Prohibited Medications Database located at http://tprc.pharm.buffalo.edu/home/di_search/.

5.5.1 Required Medications

Suppressive ART as specified in section 4.1.5.

5.5.2 Prohibited Medications

A list of prohibited medications is available on the PSWP.

5.5.3 Precautionary Medications

A list of precautionary medications is available on the PSWP.

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Evaluations (SOE)

Evaluation	Screen	Pre-Entry	Entry	Post-Entry Evaluations								Confirmation of Virologic Failure	Premature Study/Treatment Discontinuation
				Week 4	Week 5		Week 6			Week 7	Week 9		
				Day 28	Day 35		Day 38			Day 45	Day 65		
					Hour 0	Hour 0.5	Hour 0	Hour 0.5	Hour 5.5				
Documentation of HIV-1	X												
Medical History	X		X										
Medication History	X		X										
Complete Physical Exam	X				X								
Targeted Physical Exam		X	X	X			X		X	X	X	X	X
Clinical Assessments		X	X	X	X		X			X	X	X	X
ECG	X												
Hematology	X	X		X						X		X	X
Liver Function Tests	X	X		X						X		X	X
Blood Chemistries	X	X		X						X		X	X
Calculated CrCl	X	X		X						X		X	X
Pregnancy Test	X												
Hormone Testing (FSH)	X												
Hepatitis Screen	X												
CD4+/CD8+ Count	X			X						X		X	X

[illegible]

6.2 Timing of Evaluations

6.2.1 Screening and Pre-Entry Evaluations

Screening and pre-entry evaluations must occur prior to the participant starting any study medications, treatments, or interventions.

Screening

Screening evaluations to determine eligibility must be completed between 90-50 days prior to study entry unless otherwise specified.

In addition to data being collected on participants who enroll into the study, demographic, clinical, and laboratory data on screening failures will be captured on a Screening Failure Results eCRF and entered into the ACTG database.

Due to blood volume restrictions, candidates who fail screening must wait a minimum of 60 days before being rescreened.

Pre-Entry

Pre-entry evaluations must be completed between 21 and 14 days prior to entry and at least 29 days after screening evaluations have been completed.

Pre-entry laboratory values must be obtained and reviewed prior to initiating tamoxifen.

6.2.2 Entry Evaluations

Entry evaluations must occur at least 14 days after pre-entry evaluations unless otherwise specified. Participants in Arm A must begin study treatment within 3 days after randomization.

6.2.3 Post-Entry Evaluations

For Arm A, all post-entry evaluations occur in reference to the date on which the participant starts study treatment. For example, day 28 evaluations occur 28 days after the date participant started study treatment.

For Arm B, post-entry evaluations occur in reference to the date of study entry.

On-Treatment Evaluations

The day 28 visit must occur 28 days ± 3 days after entry or initiation of tamoxifen, if the latter is delayed.

The window for the day 35 visit is -3 and +2 days.

The window for the day 38 visit is -2 and +3 days.

The day 35 visit cannot overlap with the day 38 visit.

Post-Treatment Evaluations

The day 45 and day 65 visits have a window of ± 3 days for each visit.

Study Completion Evaluations

The day 65 evaluations will serve as the study completion evaluations unless a participant experiences an adverse event, in which case monitoring will be as described in section 8.

Confirmation of Virologic Failure

If the day 28 expedited HIV-1 RNA level is ≥ 200 copies/mL, the participant should be contacted immediately upon the site's receipt of the HIV-1 RNA result and asked to return for a confirmatory visit for repeat expedited HIV-1 RNA as soon as possible, optimally within 72 hours of the site's receipt of the original result. Tamoxifen should be continued during the time that confirmation is being obtained for participants in Arm A. Participants should not proceed to vorinostat dosing while awaiting HIV-1 RNA confirmation.

PK samples for vorinostat will not be collected at this confirmatory visit unless the visit occurs between day 35 and 38. PK samples for ARVs and tamoxifen (Arm A) will be collected at this study visit.

If the HIV-1 RNA is confirmed to be ≥ 200 copies/mL, the participant should not proceed to vorinostat dosing. These participants will have premature study treatment discontinuation evaluations performed as noted on the SOE and described below. These participants will remain on study, off study treatment and have all evaluations performed per the SOE.

6.2.4 Discontinuation Evaluations

Evaluations for Randomized Participants Who Do Not Start Study Treatment

All eCRFs must be keyed for the period up to and including the entry visit.

Premature Treatment Discontinuation Evaluations

Participants who prematurely permanently discontinue study treatment will have premature study treatment discontinuation evaluations performed as noted on the SOE. These participants will remain on study, off study treatment and have all evaluations performed per the SOE.

PK samples for vorinostat will not be collected at this visit unless the visit occurs between day 35 and 38. PK samples for ARVs and tamoxifen (Arm A) will be collected at this study visit.

Site personnel should notify the core protocol team within 72 hours of any participant who prematurely discontinues study treatment.

Premature Study Discontinuation Evaluations

Participants who prematurely discontinue from the study will have premature study discontinuation evaluations performed as noted on the SOE.

Site personnel should notify the core protocol team within 72 hours of any participant who prematurely discontinues the study.

6.3 Instructions for Evaluations

All clinical and laboratory information required by this protocol is to be present in the source documents. Sites must refer to the Source Document Guidelines on the DAIDS website for information about what must be included in the source document: <https://www.niaid.nih.gov/sites/default/files/sourcedocappndx.pdf>.

All stated evaluations are to be recorded on an eCRF unless otherwise specified. Refer to section 7.0 for information on the DAIDS AE Grading Table and requirements for reporting AEs.

6.3.1 Documentation of HIV-1

Section 4.1.1 specifies assay requirements for HIV-1 documentation. HIV-1 documentation is not recorded on an eCRF.

6.3.2 Medical History

The medical history must include all signs and symptoms regardless of grade within the past 30 days and all diagnoses identified by the ACTG criteria for clinical events and other diagnoses regardless of grade within the past 30 days. Diagnoses, signs & symptoms, and medical history must be recorded on eCRFs and keyed within two business days.

In addition, the following diagnoses must be recorded regardless of when the diagnosis was made:

- AIDS-defining conditions
- Bone fractures (verbal history accepted)
- Coronary heart disease
- Cancer (exclusive of basal/squamous cell skin cancer)
- Diabetes
- Tuberculosis
- Chronic Hepatitis C
- Chronic Hepatitis B
- Pregnancy
- Document the pre-ART HIV-1 RNA level and nadir CD4 count, if available (if nadir CD4 count or pre-ART HIV-1 RNA documentation is not available, then collect and record participant recall)

- Record the date of the first undetectable HIV-1 RNA level prior to sustained viral load suppression (may be estimated if exact date not available)
- Record date(s) of any previous virologic failure on ART (if known) as determined by the investigator
- If recent cessation of smoking, record date of cessation
- Document any allergies to any medications and their formulations

6.3.3 Medication History

A medication history must be present, including start and stop dates.

Table 6.3.3-1: Required Medication History

Medication Category	Complete History or Timeframe
Antiretroviral therapy	Complete history
Immune-based therapy	Complete history
Blinded study treatment	Complete history
HIV-1-related vaccines	Complete history
Prescription drugs	Within 60 days prior to entry
Sex-hormone medications or sex-hormone analogues or antagonists*	Last 12 months except as noted below
Potassium and magnesium supplements	Within 21 days prior to entry

*Includes: hormone-releasing IUDs (e.g., Mirena inserted in the last 5 years); oral, injectable, implanted, or patch contraceptives; vaginal ring, creams, or inserts; estrogen, progesterone, or testosterone therapy; leuprolide or other synthetic gonadotropin-releasing hormone; tamoxifen, raloxifene, aromatase inhibitors or any other androgen, estrogen, or progesterone analogue or antagonist therapy.

6.3.4 Complete Physical Examination

A complete physical examination must include, at a minimum, an examination of the skin, head, mouth, and neck; auscultation of the chest; cardiac examination; abdominal examination; examination of the lower extremities for edema; calculation of a Karnofsky performance score; signs and symptoms; diagnoses; height (cm); weight (kg); temperature; pulse; respiration rate; and blood pressure.

6.3.5 Targeted Physical Examination

A targeted physical examination must include, at a minimum, vital signs (height, weight, temperature, pulse, respiration rate, and blood pressure). The exam will be driven by any previously identified or new signs or symptoms and diagnoses that the participant has experienced since the last visit or at this visit.

Refer to section 7.2, Adverse Event Collection Requirements, for AE reporting requirements.

6.3.6 Clinical Assessments

Concomitant Medications

Post-entry, record all new or discontinued medications, including potassium and magnesium supplements, since last visit.

Antiretroviral Treatment Modifications

Post entry, all modifications to the participant's ARV regimen, including any ARV interruptions (there is no minimum number of days of interruption needed for report), dose modifications, formulation modifications, starts, and permanent discontinuations since the last study visit or at the study visit must be recorded on eCRFs and keyed within two business days.

Study Treatment Modifications

Post entry, record all study treatment modifications, including initial doses, participant-initiated and/or protocol-mandated modifications, and inadvertent and deliberate interruptions of more than one day since the last study visit. Record any permanent discontinuation of study treatment on eCRFs and key within two business days.

6.3.7 ECG

For the ECG done at screening, the QTc interval must be signed off by the site investigator prior to treatment with vorinostat and should ideally be taken from limb lead II. ECG results do not need to be recorded on an eCRF.

NOTE: A program for calculating QTc by Fridericia's correction is available on the DMC website at www.fstrf.org.

6.3.8 Laboratory Evaluations

At screening, pre-entry, and entry, all laboratory values and toxicities must be recorded on eCRFs and keyed at entry. For post-entry assessments, record all Grade ≥ 2 laboratory values and key within 2 business days. Refer to section 7.2 for AE reporting requirements for abnormal laboratory findings.

Hematology

Hemoglobin, hematocrit, red blood cells [RBC], mean corpuscular volume [MCV], white blood cell count [WBC], differential WBC, absolute neutrophil count (ANC), and platelets.

Liver Function Tests

Total bilirubin, AST [SGOT], ALT [SGPT], alkaline phosphatase, and direct and indirect bilirubin.

Blood Chemistries

Electrolytes [sodium, potassium, chloride, phosphate, bicarbonate], magnesium, calcium, creatinine, and blood urea nitrogen (BUN).

Blood potassium and magnesium values on day 28 must be within the site's laboratory's normal limits. If the potassium and/or magnesium value on day 28 is below the lab's limit of normal, the participant should be supplemented as medically indicated and retested as soon as possible. If the potassium or magnesium level is above the lab's limit of normal, the participant should be encouraged to increase his or her oral hydration and should be then retested as soon as possible. The repeated potassium and/or magnesium values must be within range for the lab's limit of normal for the administration of vorinostat to take place on days 35 and 38. If the potassium and magnesium levels are still out of range after retest, vorinostat should not be given and the site should consult with the core protocol team.

Calculated Creatinine Clearance

Calculated creatinine clearance estimated by the Cockcroft-Gault equation will be evaluated.

NOTE: A calculator for the Cockcroft-Gault equation is available on the DMC website at www.fstrf.org.

Pregnancy Test

Serum or urine β -HCG (urine test must have a sensitivity of ≤ 25 mIU/mL).

Hormone Testing: Follicular Stimulating Hormone

Levels of follicular stimulating hormone (FSH) will be measured as indicated on the SOE. Results will not be recorded on eCRFs.

Hepatitis Screen

HBsAg and HCV antibody testing will be done per the SOE. HCV RNA testing is required if HCV antibody positive. Results will not be recorded on eCRFs.

6.3.9 CD4+/CD8+

Obtain absolute CD4+/CD8+ count and percentages at screening from a laboratory that possesses CLIA certification or equivalent.

Post entry, absolute CD4+/CD8+ counts should be obtained again as per the SOE.

For entry and post-entry evaluations, all laboratories must possess a CLIA certification or equivalent and must be certified for protocol testing by the DAIDS Immunology Quality Assurance (IQA) Program.

6.3.10 Plasma HIV-1 RNA (Real-Time)

Screening HIV-1 RNA (real-time) by standard ultrasensitive assay must be obtained within 90 days prior to study entry. Eligibility will be determined based on the screening value.

All post-screening HIV-1 RNA quantification assays will be performed using the Roche Taqman V2.0 assay. Samples will be sent real time per the SOE to the protocol-designated laboratory.

6.3.11 Plasma HIV-1 RNA (Expedited)

Samples for day 28 HIV-1 RNA quantification and confirmation of virologic failure must be processed and shipped overnight. See LPC for shipping instructions.

6.3.12 Vorinostat Dosing

If day 28 liver function tests are elevated (Grade ≥ 3), the site must consult with the core protocol team before dosing vorinostat on day 35.

If tamoxifen is withheld/discontinued prior to day 35, vorinostat should not be administered on day 35 or day 38.

On day 35, all evaluations, including blood draws, must be done prior to administration of vorinostat. Vorinostat dosing times on the SOE (hour 0.5) are provided as a guide to ensure that all required evaluations are completed prior to administration of vorinostat.

On day 38, the first blood draw for pharmacokinetic studies must be done prior to administration of vorinostat. Vorinostat dosing times on the SOE (hour 0.5) are provided as a guide to ensure that all required evaluations are completed prior to administration of vorinostat.

On day 38, the second blood draw must be done 5 hours after administration of vorinostat.

Dehydration often occurs with administration of vorinostat. Participants should be instructed to drink at least 1 liter of water (32 ounces or 4 cups) on the days when vorinostat is administered.

6.3.13 Stored Plasma for Hormone Testing: Estradiol and Progesterone

Levels of estradiol and progesterone will be measured as indicated on the SOE.

6.3.14 Stored Plasma for HIV-1 RNA Single Copy Assay (SCA)

HIV-1 RNA by SCA will be measured in plasma obtained per the SOE.

6.3.15 Stored PBMCs for Histone Acetylation

Histone acetylation assay will be performed in total CD4+ cells isolated from PBMCs.

6.3.16 Stored Plasma & PBMCs for Immunology and Virology Assays

Plasma and PBMCs will be obtained per the SOE for immunology and virology assays. Refer to the LPC for details of collection, processing, and shipping.

Cell-Associated Total HIV-1 DNA and CA-RNA

Cell-associated total HIV-1 RNA and HIV-1 DNA (total and integrated) will be measured in CD4+ T cells from PBMCs.

CD4+ T Cells for Inducible HIV-1 RNA Expression (EDITS)

Inducible HIV-1 expression will be measured from PBMCs.

T Cell Phenotyping

PBMCs will be collected for immunologic studies, T cell subset frequencies, and markers of T cell activation, proliferation, and exhaustion.

HIV-Specific T Cell Responses

PBMCs will be collected for studies of HIV-specific T cell responses. PBMCs will be used to assess CD4+ and CD8+ T cells responses to HIV antigen.

CD4+ T Cells for Ultrasensitive p24 Antigen Quantification

Intracellular HIV-1 p24 antigen levels will be measured from PBMCs.

6.3.17 Pharmacokinetic Samples: ARVs, Tamoxifen (Arm A), and Vorinostat

PK samples as outlined in section 11.0 will be collected at the time points indicated on the SOE. Specific blood volume requirements for these time points are as per the LPC.

6.3.18 Adherence Assessment

Adherence to tamoxifen and antiretroviral therapy will be assessed by self-report at the intervals indicated on the SOE.

6.3.19 Questionnaire

A questionnaire regarding study participation, HIV cure research, and prior clinical trial participation will be administered as per the SOE. The questionnaire

will not be recorded on an eCRF; it will be completed by the participant online. Links to the survey and instructions can be found on the PSWP.

7.0 ADVERSE EVENTS AND STUDY MONITORING

7.1 Definition of Adverse Events

An adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or diagnosis that occurs in a study participant during the conduct of the study REGARDLESS of the attribution (i.e., relationship of event to medical treatment/study product/device or procedure/intervention). This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition.

7.2 Adverse Event Collection Requirements

All AEs must be reported on eCRFs and keyed within two business days if any of the following criteria have been met:

- All grade ≥ 3 AEs
- All AEs that led to a change in study treatment regardless of grade
- All AEs meeting SAE definition or EAE reporting requirement

NOTE: SAEs or events meeting EAE reporting requirements should also be entered into the DAIDS Adverse Experience Reporting System (DAERS), an Internet-based reporting system.

All AEs that are reported must have their severity graded. To grade AEs, sites must refer to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), corrected Version 2.1, July 2017, which can be found on the DAIDS RSC website at <http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables>.

Serious Adverse Events (SAEs)

An SAE is defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above)

7.3 Expedited Adverse Event (EAE) Reporting to DAIDS

7.3.1 Expedited Reporting of Adverse Events to DAIDS

Requirements, definitions and methods for expedited reporting of Adverse Events (AEs) are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the RSC website at <http://rsc.tech-res.com/clinical-research-sites/safety-reporting/manual>.

The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, must be used for EAE reporting to DAIDS. In the event of system outages or technical difficulties, EAEs may be submitted using the DAIDS EAE Form. This form is available on the DAIDS RSC website at <http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids/paper-eae-reporting>.

For questions about DAERS, please contact NIAID CRMS Support at CRMSSupport@niaid.nih.gov. Please note that site queries may also be sent from within the DAERS application itself.

For questions about expedited reporting, please contact the DAIDS RSC Safety Office at (DAIDSRSCSafetyOffice@tech-res.com).

7.3.2 Reporting Requirements for this Study

- The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study.
- The study agents for which expedited reporting are required are tamoxifen and vorinostat.
- In addition to the EAE Reporting Category identified above, other AEs that must be reported in an expedited manner are:
 - All thromboembolic events
 - All cardiac events requiring hospitalization
 - Grade ≥ 2 platelet count
 - Grade ≥ 2 hemoglobin
 - Vaginal bleeding events (see section 8.2)
 - Grade ≥ 2 nausea that is unresponsive to antiemetic therapy
 - Marked changes in glucose control defined as a change of ≥ 2 grades that is not attributable to changes in diabetes treatment regimen or adherence to diabetes treatment regimen
 - Any event requiring a change in study treatment
 - Any bone fractures
 - Any incident cancers
 - Fetal losses
 - Serious immune reconstitution inflammatory syndrome (IRIS) events

7.3.3 Grading Severity of Events

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), corrected Version 2.1, July 2017, must be used and is available on the DAIDS RSC website at <http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables>.

7.3.4 Expedited AE Reporting Period

- The expedited AE reporting period for this study is for the duration of the protocol.
- After the protocol-defined AE reporting period, unless otherwise noted, only suspected, unexpected serious adverse reactions (SUSARs), as defined in Version 2.0 of the EAE Manual, will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).
- Overdoses of vorinostat, should the study team be aware of them, do not require expedited reporting, but will be reported to Merck every three months by the Statistical and Data Analysis Center (SDAC)/DMC. Overdoses of vorinostat should be recorded on the CRF and keyed in the database in a timely manner.

7.4 Study Monitoring

The core protocol team will monitor the conduct of the study via regular summaries pooled over treatment arms of accrual, baseline characteristics, data completeness, and specimen collection. The core protocol team will review individual participant-level safety data frequently to assess the relation of all reported AEs to study treatment; core team review of AEs will be unblinded to study treatment arm. In addition, the core team will regularly review by-arm summaries of premature study discontinuations and premature study treatment discontinuations (and reasons), AEs, any missed vorinostat doses, and any reported interruptions or changes in ART and HIV-1 RNA levels ≥ 200 copies/mL (see section 6.2.3). Any interruptions in tamoxifen will also be summarized and reviewed by the core team.

The DAIDS clinical representative will review and assess EAE reports for potential impact on the study participant safety and protocol conduct as per DAIDS policies, guidance documents, and SOPs, as applicable.

The study will undergo interim review at least annually by an independent ACTG-appointed Study Monitoring Committee (SMC). The SMC will review accrual, baseline characteristics, conduct of the study (including premature study discontinuations, premature study treatment discontinuations and any reported interruptions or changes in ART), AEs by study treatment arm (including core protocol team assessment of relationship to study treatment), CD4+ T-cell counts and HIV-1 RNA levels/suppression over time by study treatment arm, and completeness of follow-up and sample availability. In particular, AEs of thromboembolic events and vaginal bleeding will be

monitored. The first interim review by the SMC will occur approximately 6 months after the enrollment of the first study participant or, if earlier, after 10 participants have enrolled and initiated study treatment. Regarding review of study implementation in terms of accrual, if the study has not enrolled 50% of the target sample size within 12 months after the first participant is enrolled (i.e., enrollment of n=15 within 12 months), this will trigger a review of the protocol by the team leadership along with SMC. This review will focus on barriers to enrollment and steps that the team and the ACTG can take to increase the pace of enrollment. Potential steps include changes to the protocol design, in particular the study eligibility criteria. If the study is not able to accrue the target population and sample size, consideration will be given to possible study termination for futility. This discussion will also include members of the Cure TSG leadership. An interim review may also be convened if a concern is identified by the DAIDS clinical representative, the study chairs, or study statisticians in consultation with the team. See also section 10.5 for statistical considerations related to interim monitoring.

Detailed plans for study monitoring will be outlined in a Study Monitoring Plan developed by the Statistical and Data Management Center (SDMC) prior to enrollment of the first participant.

8.0 CLINICAL MANAGEMENT ISSUES

8.1 Toxicity

Only toxicities related to study medications provided through the study will be considered in the toxicity management section.

The general guidelines presented in section 8.1 apply to toxicities that are not specifically addressed in section 8.2.

Grade 1 or 2

Participants who develop Grade 1 or 2 AE or toxicity felt to be related to study drug may continue study treatment at the discretion of the site investigator with close follow-up. If a participant chooses to discontinue study treatment, the site should notify the core protocol team within 72 hours. Participants will have premature study treatment discontinuation evaluations performed as noted on the SOE. These participants will remain on study, off study treatment and have all evaluations performed per the SOE.

Grade 1 or 2 symptoms including menopausal symptoms such as hot flashes, vaginal dryness, and flushing will not obligate study treatment discontinuation. Similarly, nausea, vomiting, and diarrhea responsive to supportive measures such as antiemetics will not obligate study treatment discontinuation.

Grade 3

- Participants who develop a Grade 3 AE or toxicity thought by the site investigator to be related to study drug should have study drug withheld and the site should consult with the core protocol team. The participant should be reevaluated weekly until the

AE returns to Grade ≤ 2 , at which time study drug may be reintroduced at the discretion of the site investigator in consultation with the core protocol team.

- If the same Grade 3 AE recurs within 4 weeks after reintroduction of tamoxifen, study drug must be permanently discontinued if the site investigator considers the AE related to study drug and the site should notify the core protocol team within 72 hours. However, if the same Grade 3 AE recurs after 4 weeks, the management scheme outlined above may be repeated.
- Participants experiencing Grade 3 AEs requiring permanent discontinuation of study drug should be followed weekly until resolution of the AE. Participants will have premature study treatment discontinuation evaluations performed as noted on the SOE. These participants will remain on study, off study treatment and have all evaluations performed per the SOE.

Grade 4

- Participants with Grade 4 asymptomatic laboratory abnormalities may continue study drug if the site investigator has compelling evidence that the toxicity is NOT related to study drug.
- Participants who develop a Grade 4 symptomatic AE or toxicity will have study drug permanently discontinued and the site should notify the core protocol team within 72 hours.
- Participants experiencing Grade 4 AEs requiring permanent discontinuation of study drug should be followed weekly until resolution of the AE or return to baseline. Participants will have premature study treatment discontinuation evaluations performed as noted on the SOE. These participants will remain on study, off study treatment and have all evaluations performed per the SOE.

8.2 Specific Management of Toxicities Related to Study-Provided Drugs

Virologic Failure

If the day 28 real-time HIV-1 RNA level is ≥ 200 copies/mL, the participant should be contacted immediately upon the site's receipt of the HIV-1 RNA result and asked to return for a confirmatory visit as soon as possible, optimally within 72 hours of the original result. Tamoxifen should be continued during the time that confirmation is being obtained for participants in Arm A. Participants should not proceed to vorinostat dosing while awaiting HIV-1 RNA confirmation.

If the HIV-1 RNA is confirmed to be ≥ 200 copies/mL (virologic failure), the participant should not proceed to vorinostat dosing. The site should notify the core protocol team within 72 hours.

Participants will have premature study treatment discontinuation evaluations performed as noted on the SOE. These participants will remain on study, off study treatment and have all evaluations performed per the SOE.

Vaginal Bleeding

Participants should be instructed to report vaginal bleeding. If a participant develops vaginal bleeding, the site should consult with the core protocol team, which may recommend tamoxifen discontinuation, an endometrial ultrasound, a gynecological evaluation, deferral of subsequent vorinostat administration, and/or another course of action.

Diarrhea/Nausea/Vomiting

Participants are permitted to receive supportive care measures as considered appropriate by the site investigator including:

- Diarrhea: Diarrhea should be treated promptly with appropriate supportive care per guidelines. Supportive care should begin at the first sign of poorly formed or loose stool, occurrence of more bowel movements than usual in one day, or unusually high volume of stool. Supportive care should be deferred, and evaluation per standard clinical guidelines pursued, if blood or mucus is present in the stool or if diarrhea is accompanied by fever. Participants should also be advised to drink at least 2 liters/day of clear fluids to help prevent dehydration.
- Nausea and Vomiting: Nausea and vomiting should be managed according to standard practice.

Vorinostat Dose-Limiting Toxicity

Non-hematologic dose-limiting toxicity is defined as any confirmed symptomatic Grade ≥ 3 , if related (definitely, probably, or possibly) to vorinostat therapy, with the specific exception of:

- Grade 3 vomiting or diarrhea that lasts for less than 12 hours
- Grade 3 elevation of liver function tests (LFTs), glucose, lipase, or creatinine without associated clinical symptoms, lasting for 7–14 days

For any symptoms Grade ≥ 3 reported between the two scheduled vorinostat doses, the following modifications may be considered in consultation with the core protocol team:

- Vorinostat dosing may be halted
- Laboratory testing may be performed

Any participant who does not complete the full vorinostat dosing schedule will have premature study treatment discontinuation evaluations performed as noted on the SOE. The site should notify the core protocol team within 72 hours. These participants will remain on study, off study treatment and have all evaluations performed per the SOE.

Deep Vein Thrombosis

Participants in the tamoxifen arm who develop signs or symptoms of deep vein thrombosis including local pain, swelling, erythema, or a palpable cord should be evaluated for intravascular clot with a venous ultrasound. If the presence of a deep vein thrombosis is confirmed, tamoxifen should be discontinued and vorinostat will not be administered. The site should notify the core protocol team within 72 hours. Clinical management should be as per standard of care with referral to primary care and/or vascular medicine or hematology, as appropriate. Participants will have premature study

treatment discontinuation evaluations performed as noted on the SOE. These participants will remain on study, off study treatment and have all evaluations performed per the SOE.

Malignancy

All study treatment will be discontinued and the site should notify the core protocol team within 72 hours if a participant is diagnosed with any malignancy while on study, except for skin cancers not requiring systemic treatment. Carcinoma in situ (CIS) is not considered a malignancy for the purposes of this study.

These participants will remain on study, off study treatment and have all evaluations performed per the SOE.

9.0 CRITERIA FOR DISCONTINUATION

9.1 Premature Study Treatment Discontinuation

- Requirement for prohibited concomitant medications (see section 5.4)
- Request by participant to terminate study treatment
- Clinical reasons believed life threatening by the physician, even if not addressed in the toxicity section of the protocol
- Confirmed viral load ≥ 200 copies/mL
- Discontinuation of ART

9.2 Premature Study Discontinuation

- Failure by the participant to attend two or more consecutive clinic visits
- Request by the participant to withdraw
- Request of the primary care provider if she or he thinks the study is no longer in the best interest of the participant
- Participant judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results
- At the discretion of the IRB/EC, FDA, NIAID, Office for Human Research Protections (OHRP), other government agencies as part of their duties, investigator, or industry supporter

10.0 STATISTICAL CONSIDERATIONS

10.1 General Design Issues

A5366 is a randomized, open-label, exploratory study to evaluate whether tamoxifen enhances the viral reactivation effect of vorinostat, an HDACi that previously has been shown to reactivate latent HIV. Study participants will be post-menopausal women well suppressed on ART with CD4+ T cell counts >300 cells/mm³. Each participant will receive two doses of vorinostat, with primary efficacy readout based on a blood sample

obtained 5 hours after the second dose of vorinostat. Participants randomized to the tamoxifen arm (Arm A) will additionally receive daily tamoxifen for 38 days prior to and during receipt of vorinostat. Accrual of the targeted 30 participants is anticipated to take 18 months.

10.2 Outcome Measures

10.2.1 Primary Outcome Measures

10.2.1.1 Safety

Occurrence of a new Grade ≥ 3 adverse event that is considered definitely, probably, or possibly related to study treatment (as judged by the core protocol team)

10.2.1.2 Efficacy

Change in cell-associated HIV-1 RNA in resting CD4+ T cells from baseline to the day 38 post-vorinostat time point

10.2.2 Secondary Outcome Measures

10.2.2.1 Single copy HIV-1 RNA assay

10.2.2.2 Total HIV-1 DNA levels

10.2.3 Exploratory Outcome Measures

10.2.3.1 Integrated HIV-1 DNA levels, HIV p24 antigen expression, and ex vivo HIV-1 RNA expression

10.2.3.2 HIV-specific immunity and activation: HIV-1-specific immune responses, T-cell activation, T-cell exhaustion markers

10.2.3.3 Histone H3 acetylation

10.2.3.4 Pharmacokinetic parameters of vorinostat and tamoxifen

10.2.3.5 Measures of innate cellular markers and soluble factors

10.3 Randomization and Stratification

Participants will be randomized in a 2:1 ratio to Arm A (tamoxifen and vorinostat) vs. Arm B (vorinostat alone), using permuted blocks. The rationale for the 2:1 randomization is that it provides for a greater number of participants to evaluate the safety of the combination treatment than in a 1:1 design.

10.4 Sample Size and Accrual

Regarding the assessment of safety, the sample size of 20 participants in the tamoxifen plus vorinostat arm provides >90% probability of observing a treatment-related adverse event (10.2.1.1) that would occur in 11% or more of combination treated participants.

The analysis of whether tamoxifen enhances the viral re-activation effect of vorinostat will be based on a 2-group t-test of the change from baseline in resting CD4 cell-associated HIV-1 RNA (10.2.1.2). With the pilot nature of this study, the test will be based on a 1-sided alpha of 0.1. The standard deviation (SD) of the change in \log_{10} -transformed cell-associated HIV RNA is assumed to 0.38, based on data from two longitudinal measurements in 18 virally suppressed individuals [40]. A similar SD was seen in the recent ACTG A5342 study: SD=0.40. Note that a smaller SD was observed in the single-dose vorinostat study (SD=0.28, n=8) [8], but this was based on assaying at each time point between 18-72 million resting CD4 cells obtained by leukapheresis; this study will instead obtain cells from large-volume blood draws and thus a smaller number of analyzed cells than in Archin et al. (2012) is anticipated.

With evaluable sample sizes of n=18 and n=9, respectively, in the two arms, the study will have 80% power to identify a 2.2-fold greater effect on HIV transcription as assessed by CA-RNA in the tamoxifen and vorinostat arm compared to vorinostat alone. To address the potential for early treatment or study discontinuation, or other analysis exclusions, an additional three participants will be enrolled. Hence, the study will enroll and randomize a total of 30 participants.

(To obtain 80% power for a 2.5-fold effect size, n=14 and 7 evaluable are needed. With a 1-sided alpha=0.05 test and 2.5-fold effect size, n=18 and 9 are needed to achieve 80% power.)

10.5 Data and Safety Monitoring

At SMC reviews, data will be considered as detailed in Section 7.4. There are no pre-specified stopping guidelines.

10.6 Analyses

10.6.1 Primary Analyses

For the primary safety analysis, AEs attributed to study treatment based on core protocol team review will be summarized separately for the two treatment arms. All participants who have been exposed to the study treatment will be included in this analysis. The primary safety outcome (see 10.2.1.1) will be summarized separately by treatment arm, including a 1-sided 95% confidence interval using exact binomial methods. Tolerability will be assessed by summaries of early treatment discontinuations, including reasons, separately by treatment arm.

Because the aim of this pilot study is to assess biologic activity, the primary efficacy analysis will be as-treated, limited to participants who received the full study treatment. In addition, any participant who interrupts ART or has confirmed HIV-1 RNA level ≥ 200 copies/mL between the pre- and post-treatment time points will be excluded. The primary efficacy outcome (10.2.1.2) is the change from baseline (average of the pre-entry and entry time points) to the post-vorinostat time point (5 hours following vorinostat dosing) of resting cell-associated HIV-1 RNA. The change (and the average of the pre-treatment measurements) will be done on \log_{10} -transformed measurements; results below assay limits will be set to half the lower limit prior to statistical analysis. (Only a minority of results, estimated to be 10%, are anticipated to be below assay limit; Dr. Athe Tsibris, Brigham and Women's Hospital, personal communication.) The primary analysis to assess whether tamoxifen enhances the effect of vorinostat will be based on a 2-group t-test. The level of the test for this pilot study will be at a 1-sided $\alpha=0.1$.

10.6.2 Secondary Analyses

Secondary analyses of HIV-1 persistence measures, immunologic measurements and histone acetylation will be analyzed similarly to the primary efficacy outcome (see 10.6.1) but using 2-sided α of 0.05 for each comparison. It is anticipated that measures of HIV-1 persistence will be log-transformed prior to analysis. Plots and descriptive data summaries will also be presented, by treatment arm, including 95% confidence intervals of changes from baseline to post-baseline time points. This will address, for example, the potential effects of tamoxifen alone on HIV-1 reservoir measures. Because it is anticipated that a substantial fraction of single copy HIV-1 RNA measurements will be below assay limit, changes will be evaluated using statistical methods for longitudinal censored data [41]. The proportion below assay limit, by arm, will also be summarized longitudinally.

Correlations between measurements will also be examined, in particular correlations between changes in the cell-associated RNA and changes in the other virologic and immunologic measures.

While measurements at pre-entry and entry will be averaged to assess treatment effects (see 10.6.1), supplemental descriptive analyses will summarize the biologic and measurement variability, and stability, of the virologic and immunologic measures while on stable suppressive ART (i.e., prior to treatment intervention).

Section 11.0, Pharmacology Plan, provides details of the analyses of the PK-related objectives.

11.0 PHARMACOLOGY PLAN

A5366 is designed primarily to evaluate safety of a sequential combination of 38-day tamoxifen and vorinostat treatment and its effectiveness on latent virus reactivation in women who are suppressed on ART in comparison to that of vorinostat alone.

Pharmacologic Considerations/Hypotheses:

1. Tamoxifen pharmacologic effects are due to the parent drug plus the active metabolite. Drug interactions that influence the ratio of parent/metabolite may influence the receptor blockade that is thought to contribute to viral latency.
2. Participants will be on suppressive ART regimens that may have either inductive or inhibitory effects on metabolic pathways, thus influencing the ratio of tamoxifen parent/metabolite.
3. Vorinostat is a P-glycoprotein (P-gp) inducer and tamoxifen is an inhibitor. Therefore, a pharmacokinetic interaction between vorinostat and concurrent tamoxifen may occur at the membrane transporter level.

11.1 Pharmacology Objectives

- 11.1.1. Investigate vorinostat plasma concentrations to compare pharmacokinetic parameters in participants with and without tamoxifen.
- 11.1.2. Investigate tamoxifen plasma concentrations in relation to ART regimens that participants are receiving upon entry.
- 11.1.3. Investigate pharmacodynamic associations between tamoxifen and vorinostat concentrations in a multivariate analysis.

11.2 Pharmacology Study Design

- 11.2.1 A single abbreviated plasma PK study of vorinostat on day 38 for all participants. This will include a pre-dose sample at time 0, and then a sampling at 5-hour post dosing. Dose information (date/time of the day 35 and day 38 vorinostat doses) will be captured on an eCRF.
- 11.2.2 Repeated trough samples of tamoxifen (Arm A) on days 35, 38, and 65. Dose information (date/time of last 3 doses) will be captured on an eCRF.
- 11.2.3 Antiretroviral plasma concentrations on days 0, 35, 38, and 65. Dose information (date/time of last 3 doses) will be captured on an eCRF.

11.3 Primary and Secondary Data, Modeling, and Data Analysis

- 11.3.1 Vorinostat. The intensive plasma samples will be required and used to calculate key pharmacokinetic parameters of vorinostat, including exposure, as indicated by area under the concentration curve (AUC) and clearance (Cl/F) due to its short half-life.
- 11.3.2 Tamoxifen. Since tamoxifen exhibits a prolonged half-life (5-7 days), the trough concentrations of tamoxifen will adequately represent its exposure.
- 11.3.3 Pharmacodynamics. The vorinostat pharmacokinetic estimates and tamoxifen trough concentrations will then be used in analyses to correlate with the magnitude of HIV reactivation (10.2.1.2) and for comparison of exposure between participants with and without treatment-related adverse effects (10.2.1.1).
- 11.3.4 Drug interactions. The potential drug interactions between tamoxifen and selective antiretroviral agents including elvitegravir, dolutegravir, raltegravir, and efavirenz, will be explored as well as the relationship between pharmacokinetics and changes in other biomarkers.

11.4 Anticipated Outcomes

We anticipate that participants in A5366 will enter the study on a variety of ART regimens. These participants will receive the recommended doses of tamoxifen and vorinostat, as per protocol, but there will be a wide range of individual plasma exposures of these two agents based on interpatient variability. The proposed clinical pharmacology objectives will provide additional PK data and strengthen the multivariate analysis that will help identify specific covariates that are associated with the primary outcomes as well as provide a confirmation of vorinostat-driven HIV reactivation. The pharmacokinetic interactions between tamoxifen and selected antiretroviral agents will be explored to identify potentially significant ones for further investigation.

12.0 DATA COLLECTION AND MONITORING

12.1 Records to Be Kept

Electronic case report form (eCRF) screens will be made available to sites for data entry. Participants must not be identified by name on any data submitted to the DMC. Participants will be identified by the patient identification number (PID) and study identification number (SID) provided by the ACTG DMC upon randomization.

12.2 Role of Data Management

- 12.2.1 Instructions concerning entering study data on eCRFs will be provided by the ACTG DMC. Each CRS is responsible for keying study data in a timely fashion.

12.2.2 It is the responsibility of the ACTG DMC to assure the quality of computerized data for each ACTG study. This role extends from protocol development to generation of the final study databases.

12.3 Clinical Site Monitoring and Record Availability

12.3.1 Site monitors under contract to the NIAID will visit participating clinical research sites to review the individual participant records, including consent forms, eCRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts), to ensure protection of study participants, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect sites' regulatory files to ensure that regulatory requirements are being followed and sites' pharmacies to review product storage and management.

12.3.2 The site investigator will make study documents (e.g., consent forms, drug distribution forms, eCRFs) and pertinent hospital or clinic records readily available for inspection by the local IRB, the site monitors, FDA, NIAID, OHRP, other local, US, and international regulatory entities, and the industry supporter or its designee for confirmation of the study data.

13.0 PARTICIPANTS

13.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document (Appendix I) and any subsequent modifications will be reviewed and approved by the IRB or EC responsible for oversight of the study. A signed consent form will be obtained from the participant. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant and this fact will be documented in the participant's record.

13.2 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain participant confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the ACTG, IRB/EC, FDA, NIAID, OHRP, other local, US, and international regulatory entities as part of their duties, or the industry supporter or its designee.

13.3 Study Discontinuation

The study may be discontinued at any time by the ACTG, IRB/EC, FDA, NIAID, OHRP, or other government agencies as part of their duties to ensure that research participants are protected, or by the industry supporter.

14.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by ACTG policies.

15.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the CDC and the National Institutes of Health.

All dangerous goods and materials, including diagnostic specimens and infectious substances, must be transported using packaging mandated by CFR 42 Part 72. Please refer to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

16.0 REFERENCES

1. Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997; 278(5341):1295-1300.
2. Chun TW, Stuyver L, Mizell SB, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proceedings of the National Academy of Sciences of the United States of America* 1997; 94(24):13193-13197.
3. Wong JK, Hezareh M, Gunthard HF, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 1997; 278(5341):1291-1295.
4. Strain MC, Gunthard HF, Havlir DV, et al. Heterogeneous clearance rates of long-lived lymphocytes infected with HIV: intrinsic stability predicts lifelong persistence. *Proceedings of the National Academy of Sciences of the United States of America* 2003; 100(8):4819-4824.
5. Siliciano JD, Kajdas J, Finzi D, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nature Medicine* 2003; 9(6):727-728.
6. Deeks SG. HIV: Shock and kill. *Nature* 2012; 487(7408):439-440.
7. Mbonye U, Karn J. Transcriptional control of HIV latency: cellular signaling pathways, epigenetics, happenstance and the hope for a cure. *Virology* 2014; 454-455:328-339.
8. Archin NM, Liberty AL, Kashuba AD, et al. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* 2012; 487(7408):482-485.
9. Elliott JH, Wightman F, Solomon A, et al. Activation of HIV transcription with short-course vorinostat in HIV-infected patients on suppressive antiretroviral therapy. *PLoS Pathogens* 2014; 10(10):e1004473.
10. Rasmussen TA, Tolstrup M, Moller HJ, et al. Activation of latent human immunodeficiency virus by the histone deacetylase inhibitor panobinostat: a pilot study to assess effects on the central nervous system. *Open Forum Infectious Diseases* 2015; 2(1):ofv037.
11. Wei DG, Chiang V, Fyne E, et al. Histone deacetylase inhibitor romidepsin induces HIV expression in CD4 T cells from patients on suppressive antiretroviral therapy at concentrations achieved by clinical dosing. *PLoS Pathogens* 2014; 10(4):e1004071.
12. Sogaard OS, Graversen ME, Leth S, et al. The Depsipeptide Romidepsin Reverses HIV-1 Latency in Vivo. *PLoS Pathogens* 2015; 11(9):e1005142.

13. Spivak AM, Andrade A, Eisele E, et al. A pilot study assessing the safety and latency-reversing activity of disulfiram in HIV-1-infected adults on antiretroviral therapy. *Clinical Infectious Diseases* 2014; 58(6):883-890.
14. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol* 2016; 16(10):626-638.
15. Markle JG, Fish EN. Sex matters in immunity. *Trends Immunol* 2014; 35(3):97-104.
16. Johnston RE, Heitzeg MM. Sex, age, race and intervention type in clinical studies of HIV cure: a systematic review. *AIDS Research and Human Retroviruses* 2015; 31(1):85-97.
17. Ho YC, Shan L, Hosmane NN, et al. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* 2013; 155(3):540-551.
18. Das B, Dobrowolski C, Shahir AM, et al. Short chain fatty acids potentially induce latent HIV-1 in T-cells by activating P-TEFb and multiple histone modifications. *Virology* 2015; 474:65-81.
19. Mirkin S, Pickar JH. Selective estrogen receptor modulators (SERMs): a review of clinical data. *Maturitas* 2015; 80(1):52-57.
20. Munster PN, Thurn KT, Thomas S, et al. A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. *British Journal of Cancer* 2011; 104(12):1828-1835.
21. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022393s000_PharmR_P1.pdf.
22. de Vries Schultink AH, Zwart W, Linn SC, Beijnen JH, Huitema AD. Effects of Pharmacogenetics on the Pharmacokinetics and Pharmacodynamics of Tamoxifen. *Clin Pharmacokinet* 2015; 54(8):797-810.
23. Antoniou T, Tseng AL. Interactions between antiretrovirals and antineoplastic drug therapy. *Clin Pharmacokinet* 2005; 44(2):111-145.
24. Binkhorst L, van Gelder T, Loos WJ, et al. Effects of CYP induction by rifampicin on tamoxifen exposure. *Clin Pharmacol Ther* 2012; 92(1):62-67.
25. Hariparsad N, Nallani SC, Sane RS, Buckley DJ, Buckley AR, Desai PB. Induction of CYP3A4 by efavirenz in primary human hepatocytes: comparison with rifampin and phenobarbital. *J Clin Pharmacol* 2004; 44(11):1273-1281.

26. Cotreau MM, von Moltke LL, Harmatz JS, Greenblatt DJ. Molecular and pharmacokinetic evaluation of rat hepatic and gastrointestinal cytochrome p450 induction by tamoxifen. *Pharmacology* 2001; 63(4):210-219.
27. Desai PB, Nallani SC, Sane RS, et al. Induction of cytochrome P450 3A4 in primary human hepatocytes and activation of the human pregnane X receptor by tamoxifen and 4-hydroxytamoxifen. *Drug Metab Dispos* 2002; 30(5):608-612.
28. Zhao XJ, Jones DR, Wang YH, Grimm SW, Hall SD. Reversible and irreversible inhibition of CYP3A enzymes by tamoxifen and metabolites. *Xenobiotica* 2002; 32(10):863-878.
29. Dowsett M, Pfister C, Johnston SR, et al. Impact of tamoxifen on the pharmacokinetics and endocrine effects of the aromatase inhibitor letrozole in postmenopausal women with breast cancer. *Clin Cancer Res* 1999; 5(9):2338-2343.
30. Jefferson JW. Tamoxifen-associated reduction in tricyclic antidepressant levels in blood. *J Clin Psychopharmacol* 1995; 15(3):223-224.
31. http://hivclinic.ca/main/drugs_interact_files/Chemo-int.pdf.
32. Iusuf D, Teunissen SF, Wagenaar E, Rosing H, Beijnen JH, Schinkel AH. P-glycoprotein (ABCB1) transports the primary active tamoxifen metabolites endoxifen and 4-hydroxytamoxifen and restricts their brain penetration. *J Pharmacol Exp Ther* 2011; 337(3):710-717.
33. Badros A, Burger AM, Philip S, et al. Phase I study of vorinostat in combination with bortezomib for relapsed and refractory multiple myeloma. *Clin Cancer Res* 2009; 15(16):5250-5257.
34. Ramalingam SS, Parise RA, Ramanathan RK, et al. Phase I and pharmacokinetic study of vorinostat, a histone deacetylase inhibitor, in combination with carboplatin and paclitaxel for advanced solid malignancies. *Clin Cancer Res* 2007; 13(12):3605-3610.
35. Rubin EH, Agrawal NG, Friedman EJ, et al. A study to determine the effects of food and multiple dosing on the pharmacokinetics of vorinostat given orally to patients with advanced cancer. *Clin Cancer Res* 2006; 12(23):7039-7045.
36. Balliet RM, Chen G, Gallagher CJ, Dellinger RW, Sun D, Lazarus P. Characterization of UGTs active against SAHA and association between SAHA glucuronidation activity phenotype with UGT genotype. *Cancer Res* 2009; 69(7):2981-2989.
37. Sandhu P, Andrews PA, Baker MP, et al. Disposition of vorinostat, a novel histone deacetylase inhibitor and anticancer agent, in preclinical species. *Drug Metab Lett* 2007; 1(2):153-161.

38. Hauswald S, Duque-Afonso J, Wagner MM, et al. Histone deacetylase inhibitors induce a very broad, pleiotropic anticancer drug resistance phenotype in acute myeloid leukemia cells by modulation of multiple ABC transporter genes. *Clin Cancer Res* 2009; 15(11):3705-3715.
39. Lautz TB, Jie C, Clark S, et al. The effect of vorinostat on the development of resistance to doxorubicin in neuroblastoma. *PLoS One* 2012; 7(7):e40816.
40. Hong F, Aga E, Cillo AR, et al. Novel Assays for Measurement of Total Cell-Associated HIV-1 DNA and RNA. *J Clin Microbiol* 2016; 54(4):902-911.
41. Vaida F, Liu L. Fast implementation for normal mixed effects models with censored response. *J Comput Graph Stat* 2009; 18:797-817.

APPENDIX I: SAMPLE INFORMED CONSENT

DIVISION OF AIDS
AIDS CLINICAL TRIALS GROUP (ACTG)
PROTOCOL A5366

Selective Estrogen Receptor Modulators to Enhance the Efficacy of Viral Reactivation with Histone Deacetylase Inhibitors, FINAL Version 1.0, 12/8/17

SHORT TITLE FOR THE STUDY: Effects of SERM and HDACi on HIV-1 Latency and RNA

INTRODUCTION

You are being asked to take part in this research study because you are a woman who is infected with HIV, the virus that causes AIDS, and you are currently taking antiretrovirals (ARVs) to treat your HIV infection. This study is sponsored by the National Institutes of Health (NIH). The doctor in charge of this study at this site is: (insert name of Principal Investigator). Before you decide if you want to be a part of this study, we want you to know about the study.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to take part in this study, you will be asked to sign this consent form. You will get a copy to keep.

WHY IS THIS STUDY BEING DONE?

ARVs are effective for treating HIV, but it is impossible to completely eliminate HIV infection from the body because some virus “hides” in the cells of the immune system. These cells with “hidden” virus are called latent immune cells. If a person stops taking ARVs, the virus in these latent immune cells makes more copies of itself and levels of HIV virus in the blood increase. Some investigational medications (these are medications not approved by the US Food and Drug Administration [FDA]) have been shown to wake up these latent cells so that they can expel the virus that is hiding there, but these medications have only been partially effective and the results are not the same in all people. Early studies suggest that these medications may not work as well for women as they do for men due to women’s hormones.

This study will look at a medication (vorinostat) that has been shown to wake up latent immune cells. Vorinostat will be given with an approved medication (tamoxifen) that blocks the hormone estrogen. Researchers will study whether these two medications given together are safe and tolerable. Researchers will also look at whether the two drugs work better together in waking up the latent virus in women than vorinostat given alone. Because Vorinostat is not an approved medication, taking it is considered experimental.

WHAT DO I HAVE TO DO IF I AM IN THIS STUDY?

Screening

If you would like to be in this study, after you have read and signed this consent form, you will come to the clinic for a screening visit to make sure you meet the requirements for joining the study. This will take about 1 hour. At this visit:

- You will have a physical exam and answer questions about your medical history and any medications you are taking or have taken in the past.
- You will have approximately 2 tablespoons of blood drawn. This blood will be used for the following tests:
 - For routine lab tests for safety and to see if you are infected with hepatitis B and/or C virus (infections of the liver).
 - To measure the amount of HIV in your blood and to measure your CD4+ cell count (cells that help fight infection).
- You will be asked to give blood (1 teaspoon) or a urine sample for a pregnancy test.
- If your HIV status is unknown or not documented, additional blood may be drawn to confirm your HIV status. You may have to sign a separate consent form for this test.
- You will have an electrocardiogram to look at the electrical activity of your heart.

Pre-Entry

If the tests at screening show that you are eligible for the study, you will have one more study visit before you join the study. This visit will take about 1 hour. At this visit:

- You will have a physical exam.
- You will have approximately 5 tablespoons of blood drawn. This blood will be used for the following tests:
 - For routine lab tests for safety.
 - Some of the blood will be stored for future protocol-required testing.
- You will answer questions about how well you take your antiretroviral (ARV) medications.

If you enter the study

At the study entry visit, you will be assigned to one of two treatment groups:

Group A:

Days 0-38: Tamoxifen 20 mg by mouth once a day

Day 35: Vorinostat 400 mg PO (vorinostat will be given by study staff at the visit)

Day 38: Vorinostat 400 mg PO (vorinostat will be given by study staff at the visit)

Group B:

Days 0-38: Observation period (no tamoxifen)

Day 35: Vorinostat 400 mg PO (vorinostat will be given by study staff at the visit)

Day 38: Vorinostat 400 mg PO (vorinostat will be given by study staff at the visit)

Your assignment is random, like the flip of a coin. You will have a chance of being in each of the two groups, but you are more likely to be in Group A because more people will be assigned to that group. You will not be able to choose your group, but you and the study doctor, as well as the study staff, will know which group you are in.

Regardless of which group you are assigned to, you will continue to take your ARV medications. ARV medications will not be provided by the study.

Entry

After your screening and pre-entry visits, you will come in for an entry visit. This visit will take about 2 hours. At this visit:

- You will have a physical exam and answer questions about your medical history and any medications you are taking or have taken in the past.
- You will have approximately 7 tablespoons of blood drawn. This blood will be used for the following tests:
 - To test the level of hormones in your blood. You will be given the results of this test.
 - To measure the amount of HIV in your blood. You will be given the results of this test.
 - For pharmacokinetic testing (to see how the levels of the study drugs rise and fall in your blood over time). You will not receive the results of this testing.
 - Some of the blood will be stored for future protocol-required testing. You will not receive the results of this testing.
- You will answer questions about how well you take your ARVs.
- You will answer a questionnaire about why you chose to enroll in this study, your understanding of HIV cure research, and whether you have participated in clinical trials before. This questionnaire will take about 45 minutes to complete.

Study Visits

After your entry visit, you will come to the clinic at days 28, 35, 38, 45, and 65. These study visits will last about 1 hour, unless noted otherwise. At these visits:

- You will have a physical exam and answer questions about any medications you are taking.
- You will have approximately 1-10 tablespoons of blood drawn, depending on the study visit. This blood will be used for the following tests:
 - To test the level of hormones in your blood (days 28, 38, 45, and 65 only). You will be given the results of these tests.
 - For routine lab tests for safety (days 28 and 45 only). You will be given the results of these tests.
 - To measure the amount of HIV in your blood (days 28 and 35 only). You will be given the results of these tests.
 - To measure your CD4+ cell count (days 28 and 45 only). You will be given the results of these tests.
 - For pharmacokinetic testing (days 35, 38, and 65 only). On day 38 your two blood samples will be taken 5.5 hours apart, so this clinic visit might take longer than usual. You will not be given the results of these tests.
 - Some of the blood will be stored for future protocol-required testing. You will not be given the results of these tests.
- You will be given your dose of vorinostat to take while you are at the clinic (days 35 and 38 only). You will be given a snack at the clinic to take with your vorinostat dose.
- You will answer questions about how well you take your ARVs and your tamoxifen (if you are in Group A).

- You will answer a questionnaire about why you chose to enroll in this study, your understanding of HIV cure research, and whether you have participated in clinical trials before (day 65 only). This questionnaire will take about 30 minutes to complete.

Additional Visits

- If your viral load shows that your anti-HIV drugs are not fighting your HIV infection well, you will come to the clinic for an additional visit.
- If you have to stop taking the study drugs early or you have to stop the study early, you will come to the clinic for an additional visit.

These visits will take about 1 hour. At these visits:

- You will have a physical exam and answer questions about any medications you are taking.
- You will have approximately 7 tablespoons of blood drawn. This blood will be used for the following tests:
 - For routine lab tests for safety. You will be given the results of these tests.
 - To test the level of hormones in your blood. You will be given the results of these tests.
 - To measure the amount of HIV in your blood and to measure your CD4+ cell count. You will be given the results of these tests.
 - For pharmacokinetic testing. You will not be given the results of these tests.
 - Some of the blood will be stored for future protocol-required testing. You will not be given the results of these tests.

If you do not enroll into the study

If you decide not to take part in this study or if you do not meet the eligibility requirements, we will still use some of your information. As part of this screening visit, some demographic (for example, age, gender, race), clinical (for example, disease condition, diagnosis), and laboratory (for example, CD4 cell count, viral load) information is being collected from you so that ACTG researchers may help determine whether there are patterns or common reasons why people do not join a study.

Other

If you agree, some of your blood that is left over after all required study testing is done may be stored (with usual protections of your identity) and used for future ACTG-approved research that is separate from this study. Genetic testing will not be done on these blood samples. These samples may be stored for an indefinite period. Results of testing performed on these samples will not be given to you. You may withdraw your consent for research on stored specimens at any time and the specimens will be discarded. No matter what you decide, it will not affect your participation in this study.

_____ YES, I agree to have my leftover blood stored.

_____ NO, I do not agree.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

About 30 women will take part in this study (20 in Group A and 10 in Group B).

HOW LONG WILL I BE IN THIS STUDY?

You will be in this study for about 65 days.

WHY WOULD THE DOCTOR TAKE ME OFF THIS STUDY EARLY?

The study doctor may need to take you off the study early without your permission if:

- the study is stopped or cancelled
- you are not able to attend the study visits as required by the study
- you stop taking your ARV medications
- the doctor believes the study is no longer in your best interest

The study doctor may also need to take you off the study drugs without your permission if:

- continuing the study drugs may be harmful to you
- you need a treatment that you may not take while on the study
- you are not able to take the study drugs as required by the study

If you must stop taking the study drugs before the study is over, the study doctor will ask you to continue to be part of the study and return for some study visits and procedures.

IF I HAVE TO PERMANENTLY STOP TAKING STUDY-PROVIDED DRUGS OR ONCE I LEAVE THE STUDY, HOW WOULD DRUGS BE PROVIDED?

During the study

If you must permanently stop taking study-provided drugs before your study participation is over, the study staff will discuss other options that may be of benefit to you.

After the study

After you have completed your study participation, the study will not be able to continue to provide you with drugs you received on the study. If continuing to take these or similar drugs/agents would be of benefit to you, the study staff will discuss how you may be able to obtain them.

WHAT ARE THE RISKS OF THE STUDY?

The drugs used in this study may have side effects, some of which are listed below. Please note that these lists do not include all the side effects seen with these drugs. These lists include the more serious or common side effects with a known or possible relationship. It is very important

that you tell your study doctor of any changes in your medical condition while taking part in the study. At any time during the study, if you believe you are experiencing any of these side effects, you have the right to ask questions on possible and /or known risks.

Risks of Vorinostat

Vorinostat comes as a capsule to take by mouth. It will be given to you with food. We will be with you when you take this medicine. Do not crush or open a vorinostat capsule. Swallow the pill whole. The medicine inside the capsule can be dangerous if it gets in your eyes, mouth, or nose, or on your skin. If contact occurs, wash your skin with soap and water or rinse your eyes thoroughly with plain water.

The risks associated with vorinostat have a greater chance of occurring when vorinostat is taken every day over several weeks. In this study, you will receive two doses of 400 mg vorinostat. Your total dose will be 800 mg. In previous studies done with cancer patients who took a single high dose (800 mg) of vorinostat, the drug was generally well tolerated. No serious clinical or laboratory abnormalities were reported and no patient had to leave the study because of a bad side effect caused by vorinostat.

The most common side effects seen with this one time dose:

- Nausea (10%)
- Fatigue (9%)

The most serious side effects observed with multiple doses of vorinostat taken every day for many weeks are:

- The development of blood clots in the legs (deep vein thrombosis)
- The development of blood clots in the lungs or pulmonary emboli (4 out of 86 patients or 4.7%)
- Squamous cell carcinoma (3 out of 86 patients or 3.5%)

Additional side effects were seen when people were given multiple doses of vorinostat every day for many weeks. These included:

- Nausea, fatigue, and diarrhea (40-50%)
- Loss of appetite
- Dehydration
- Vomiting
- Low red blood cell counts or anemia (2 out of 86 patients or 2.3%)
- Low platelet counts
- Weight loss (10-20%)
- Decreased kidney function
- Altered taste
- Increase in blood sugar
- Constipation (10% or less)

Although some of the serious side effects noted above occurred in a study population with cancers, we still need to let you know this information. We think you are unlikely to experience these side effects with the two doses of vorinostat that you will receive in this study.

We do not know if the following events are related to taking vorinostat but some single events occurring after taking vorinostat are: cholecystitis (gall bladder inflammation), death (of unknown cause), deep vein thrombosis (blood clot), enterococcal (bacterial) infection, exfoliative dermatitis (scaly skin), gastrointestinal hemorrhage (bleeding in the stomach or intestine), infection, lobar pneumonia, myocardial infarction (heart attack), ischemic stroke (stroke caused by a clot), pelvi-ureteric obstruction, sepsis (blood infection), spinal cord injury, streptococcal bacteremia (bacterial infection in the blood), fainting, T-cell lymphoma, thrombocytopenia (low platelet count), and ureteric obstruction (obstructions in the tubes that drain urine from the kidneys to the bladder).

Vorinostat has the potential to be “genotoxic”, that is, it could cause damage to your genes that might increase your risk for developing cancer.

Dehydration often occurs when you take this medicine. You will need to drink at least 1 liter of water (32 ounces or 4 cups) on the days that you are receiving the drug to keep from getting dehydrated.

Risks of Tamoxifen

Tamoxifen comes as a tablet that you should swallow whole, with water or another non-alcoholic drink. You may take tamoxifen with or without food but you should take it at about the same time each day.

Although study doctors think it is unlikely, tamoxifen may decrease some the levels of ARV medications in your blood.

The following serious side effects have been associated with the use of tamoxifen. The side effects listed below are more common in people who have received longer courses of tamoxifen than will be given in this study. If you have any of the following signs or symptoms contact your health care provider right away.

Changes in lining (endometrium) or body of your uterus (womb). These changes may be due to serious problems such as cancer of the uterus. If you have these changes, you may notice:

- Vaginal bleeding or bloody discharge that could be a rusty or brown color
- Pain or pressure in your pelvis (below your belly button)

Blood clots. These can occur in your veins or lungs or can cause a stroke. These may be serious and can cause death. You may get clots up to 2-3 months after you stop taking tamoxifen. If you have a blood clot, you may notice:

- Sudden chest pain
- Shortness of breath
- Coughing up blood
- Pain, tenderness, or swelling in one or both of your legs

Stroke. If you are having a stroke, you may notice:

- Sudden weakness, tingling, or numbness in your face, arm, or leg, especially on one side of your body
- Sudden confusion, trouble speaking or understanding
- Sudden trouble seeing in one or both eyes
- Sudden trouble walking, dizziness, loss of balance or coordination
- Sudden severe headache with no known cause

Cataracts or increased chance of needing cataract surgery. If you are developing cataracts, you may notice slow blurring of your vision.

Liver problems. If you are developing liver problems, you may notice:

- Yellowing of the skin or whites of your eyes
- Dark urine
- Pain on the right side of your stomach
- Loss of appetite, upset stomach, or vomiting
- Pale colored stools
- Itchy skin

Additional side effects may include:

- Difficulty urinating or a decrease in the amount of urine
- Hot flashes
- Noisy, rattling breathing
- Redness of the face, neck, arms and, occasionally, upper chest
- Skin changes
- Swelling of the fingers, hands, feet, or lower legs
- Troubled breathing at rest
- Weight gain or loss
- Bone pain
- Stomach cramps
- Feeling sad or difficulty concentrating

Risks of Non-Study Medications

There is a risk of serious and/or life-threatening side effects when non-study medications are taken with the study drugs. For your safety, you must tell the study doctor or nurse about all medications you are taking before you start the study and before starting any new medications while on the study. You must also tell the study doctor or nurse before enrolling in any other clinical trials while on this study.

Risks of Drawing Blood

Taking blood may cause some discomfort, bleeding, bruising, and/or swelling where the needle enters the body, lightheadedness, and in rare cases, fainting or infection.

Risks of Electrocardiogram

You may experience mild irritation, slight redness, and itching on your skin where the electrodes from the electrocardiogram machine are placed.

Risks of Social Harm

It is possible that participating in this study will make it difficult for you to keep your HIV status secret from people close to you. This may lead to unwelcome discussions about or reactions to your HIV status. Please talk with the study staff if you are worried about this.

Risks of Stored Samples

There is a risk that your stored samples and/or health information may be misused. There are laws against this kind of misuse, but they may not fully protect you. The chance that this will happen is considered small because of the security taken with your samples and information.

Risks to Future Study Participation

If you participate in this study you may not be able to participate in future studies because you have taken tamoxifen and/or vorinostat.

ARE THERE RISKS RELATED TO PREGNANCY?

Vorinostat and tamoxifen are not safe for unborn babies. You can only participate in this study if you are postmenopausal (you no longer have a menstrual period) at the time of study entry and if you agree not to participate in assisted reproductive technology in the future.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

You will receive no benefit from being in this study. Information learned from this study may help others who have HIV.

WHAT OTHER CHOICES DO I HAVE BESIDES THIS STUDY?

Instead of being in this study you have the choice of:

- treatment with prescription drugs available to you
- treatment with experimental drugs, if you qualify
- no treatment

Please talk to your doctor about these and other choices available to you. Your doctor will explain the risks and benefits of these choices.

WHAT ABOUT CONFIDENTIALITY?

We will do everything we can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, we have gotten a Certificate of Confidentiality

from the US Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. Any publication of this study will not use your name or identify you personally.

People who may review your records include the US Food and Drug Administration, the ACTG, Office for Human Research Protections (OHRP) or other local, US, and international regulatory entities as part of their duties, (insert name of site) institutional review board (IRB) (a committee that protects the rights and safety of participants in research), US National Institutes of Health (NIH), study staff, study monitors, the drug company supporting this study, and their designees. Having a Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

A description of this clinical trial will be available on www.ClinicalTrials.gov as required by US law. This web site will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site at any time.

WHAT ARE THE COSTS TO ME?

Taking part in this study may lead to added costs to you and your insurance company. In some cases, it is possible that your insurance company will not pay for these costs because you are taking part in a research study.

WILL I RECEIVE ANY PAYMENT?

[Insert site-specific information about payment]

WHAT HAPPENS IF I AM INJURED?

If you are injured as a result of being in this study, you will be given immediate treatment for your injuries. The cost for this treatment will be charged to you or your insurance company. There is no program for compensation either through this institution or the National Institutes of Health. You will not be giving up any of your legal rights by signing this consent form.

WHAT ARE MY RIGHTS AS A RESEARCH PARTICIPANT?

Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. Your decision will not have any impact on your participation in other studies conducted by NIH and will not result in any penalty or loss of benefits to which you are otherwise entitled.

We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. If you want the results of the study, let the study staff know.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study or a research-related injury, contact:

- name of the investigator or other study staff
- telephone number of above

For questions about your rights as a research participant, contact:

- name or title of person on the Institutional Review Board (IRB) or other organization appropriate for the site
- telephone number of above

SIGNATURE PAGE

If you have read this consent form (or had it explained to you), all your questions have been answered and you agree to take part in this study, please sign your name below.

Participant's Name (print)

Participant's Signature and Date

Study Staff Conducting
Consent Discussion (print)

Study Staff's Signature and Date

Witness's Name (print)
(As appropriate)

Witness's Signature and Date